United States
Department of
Agriculture

Agricultural
Research
Service

March 1995

U.S. Dairy Forage Research Center 1994 Research Summaries

U.S. Dairy Forage Research Center. 1995. U.S. Dairy Forage Research Center 1994 Research Summaries. U.S. Department of Agriculture, Agricultural Research Service, 116 pp.

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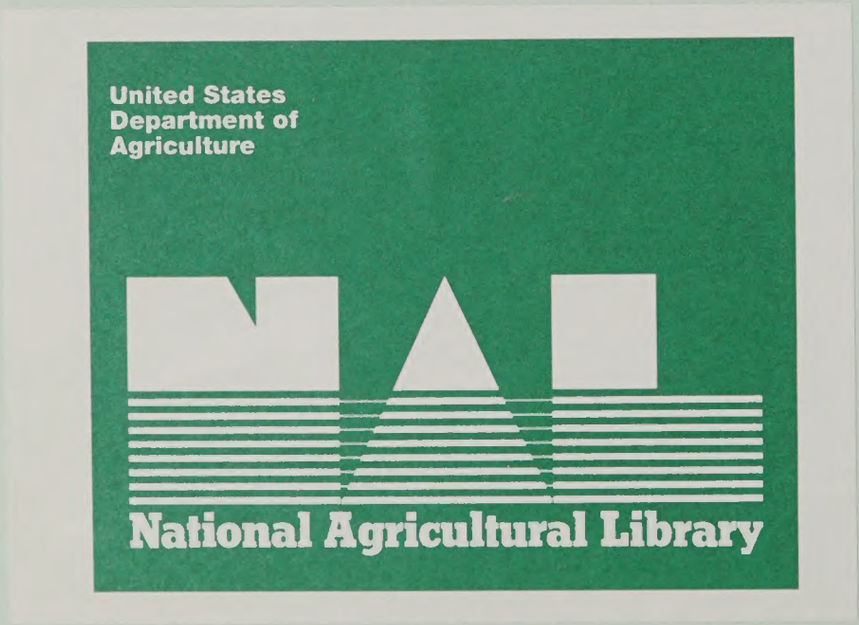
Appreciation is expressed to Gloria Palmer for her interest in and dedication to the task of typing and assembling this annual research summary.

Issued March 1995



Contents

FORAGE HARVEST AND HANDLING

FORAGE PRESERVATION AND STORAGE

PLANT CHEMISTRY

FORAGE QUALITY

RUMEN MICROBIOLOGY

FEED UTILIZATION BY CATTLE

It is a pleasure to update our progress by bringing you these summaries of recent research. The U.S. Dairy Forage Research Center is a unique part of the national research program of the Agricultural Research Service, U.S. Department of Agriculture. The Center's mission is to build a knowledge and technology base for the dairy industry to fully exploit the use of forages in the production of milk. The Center has agricultural engineers, plant and soil scientists, microbiologists, ruminant nutritionists and a chemist working together to increase the efficiency of forage production and utilization by dairy farmers. We function in close cooperation with the Agricultural Experiment Stations of several states. The Center is located on the campus of the University of Wisconsin, Madison, and has "Cluster" locations in St. Paul, MN, Ames, IA, East Lansing, MI, and Ithaca, NY. The Center's research farm with facilities for 300 milking cows is located on 63 acres of USDA land on the banks of the Wisconsin River in Prairie du Sac, WI. An additional 1200 acres of adjacent land is utilized by the Center by agreement with the U.S. Department of the Army. The Center was established in 1980 and has made steady growth since. At present there are fifteen scientists: ten at Madison, and one at each of three Cluster locations, and two at the St. Paul, Minnesota Cluster location. Scientists hold faculty appointments in university departments and provide supervision for approximately 15-20 graduate students and 5-10 post doctoral fellows.

Last year in this report we highlighted progress of the forage mat technology developed at the U.S. Dairy Forage Research Center. We are pleased that this practice of extensive conditioning (maceration), which increases the rate of field wilting and improves digestibility and animal performance, continues to progress towards commercialization. This year in our report we are highlighting the development of DAFOSYM, a dairy forage systems model. DAFOSYM is the focal point of the systems work group within the USDFRC, and we feel it is a tool that can be very useful in making strategic management decisions on the dairy farm.

The dynamics within the dairy industry that are squeezing profitability of dairy producers also impact the operating costs of dairy research facilities. Research facilities, of course, don't function to make a profit, nor do they come close to breaking even with the revenues generated. However, level milk prices for 15 years combined with increased costs of labor, supplies and capital equipment diminish the resources available for doing research with dairy cows. Research is a labor intensive endeavor. We are undertaking a self-study of our research farm this year, doing some long range planning on how we may maintain our research capability within an environment of shrinking resources. We have an excellent staff at our research farm, and they are up to the task ahead.

We are pleased and very proud of the way Center scientists from diverse disciplines interact and bring their collective insights to bear on the problems of forage production and utilization. This collection of research summaries illustrates the progress they are making in developing information to help dairy farmers utilize their resources more effectively. The research is intended to benefit producers of forage crops, dairy farmers and the consumers of dairy products.

Sincerely,

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Evaluating Technology for the Dairy Farm

Quantifying the costs and benefits of alternative technology for dairy farms is not a simple task. Technology that performs well under one set of crop and weather conditions may not perform well at other times. Long term studies are needed to quantify the benefits and costs over a wide range of conditions. Field studies of this type are costly, impractical and perhaps impossible. Another approach is to use computer simulation. Models developed and validated with limited experimental work can be used to study system performance over many years of weather. DAFOSYM, the dairy forage system model, was created for this purpose. Many alternative technologies and management strategies have been modeled with DAFOSYM to determine their long term performance and economic value to dairy farmers and other forage producers.

DAFOSYM's History

The development of DAFOSYM began in the late 70's when the U.S. Dairy Forage Research Center was initiated. The model has evolved with the development of the Center and the advancement of computer technology. The original model was developed as a joint effort between the East Lansing Cluster of the USDFRC and the faculty of Michigan State University. The East Lansing Cluster has continued to be the primary site for model development, but as the Center has grown, much input has come from the Madison lab and other Cluster programs. The first model was written for execution on a large main frame computer. With the wide acceptance of personal computers in the mid 80's, DAFOSYM was reformatted for this platform. An overlaying menu type user interface was also added to promote wider use of the model.

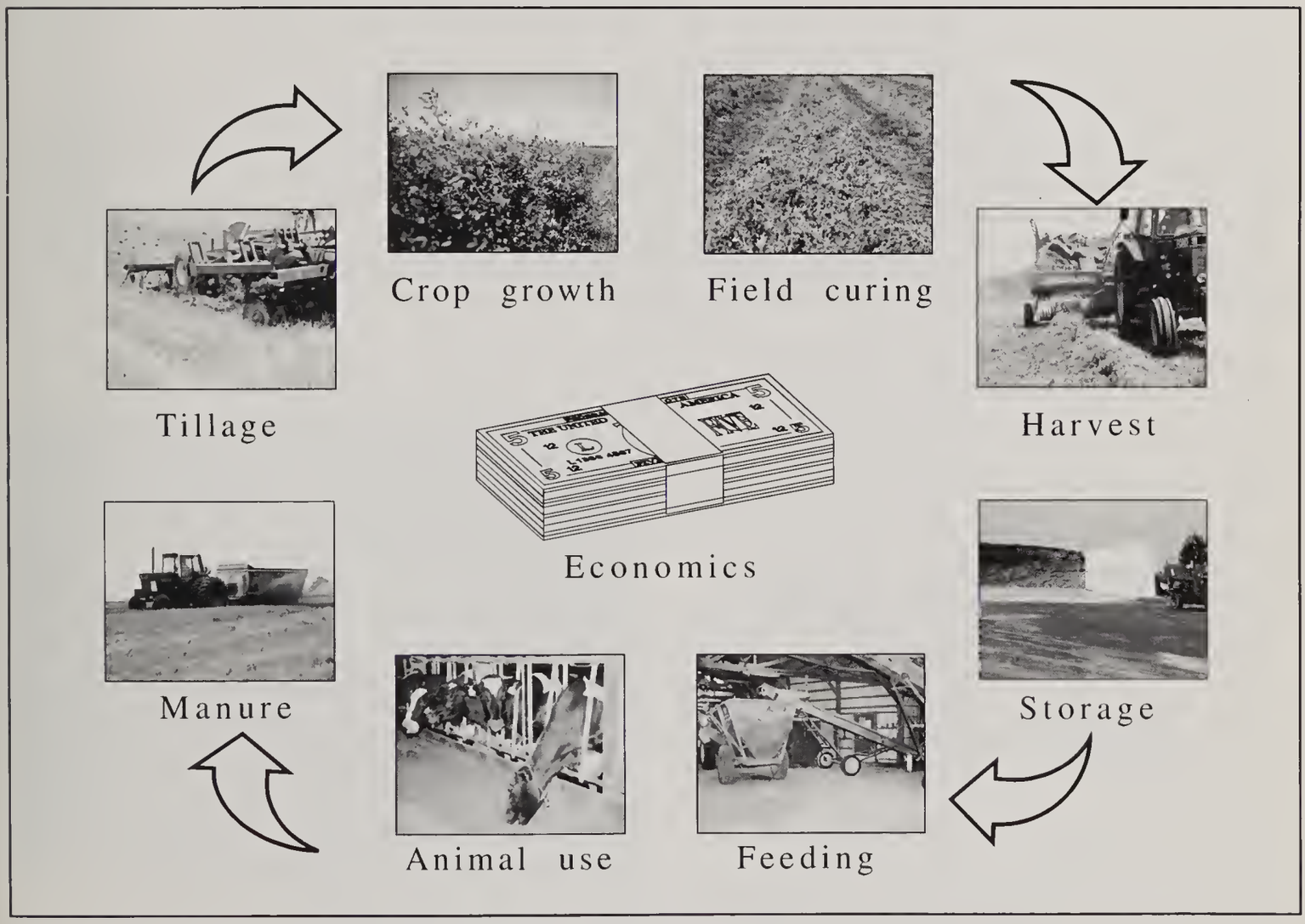


Figure 1. DAFOSYM: a simulation model of the dairy forage system.

In the early 90's, a multidisciplinary systems group was formally established in the Center to further the development of DAFOSYM and other models useful to the dairy industry. Through a Regional Research Project, NE 132, cooperative projects are also underway with scientists and engineers at the Pennsylvania State University, Washington State University and several other institutions. Current DAFOSYM projects include better utilization of nitrogen in the farm nutrient cycle, evaluation of grazing systems, and the role of grass forage crops on dairy farms in various locations of the U.S.

About DAFOSYM

DAFOSYM is a simulation model of the dairy forage system. The model simulates alfalfa and corn growth, harvest, storage, feeding and use on dairy farms (Figure 1). Also included are manure production, nutrient losses, manure handling, tillage and crop planting. The dairy forage system is simulated for many years of weather to determine long term performance and economics of alternative technologies and/or management strategies. By modeling several alternatives on the same representative farms, those alternatives which maximize farm production or profit are determined. The model is adapted to different locations by modifying weather and soil parameters.

Crop growth submodels predict yield and quality throughout the growing season. When crops are harvested, losses due to machine operations, plant respiration and rain damage are accounted to predict the quantity and quality of feed stored. Storage losses and associated quality changes are predicted for dry hay and silage stored by different methods. Following storage, feeds are either sold or allocated to a dairy herd. When used on the farm, balanced diets are fed to several animal groups with higher quality forage fed to high producing animals. Supplemental feeds are purchased as needed and extra feeds are sold. The quantity and nutrient content of manure produced is a function of feed composition and consumption, milk production and animal growth. Nutrient losses in manure handling, storage and application are subtracted to determine the nutrients available for crop growth.

Throughout the simulation, costs for machinery, structures, fuel, labor, chemicals and other inputs are totaled along with the value of feeds sold to predict production and total feed costs. Feed costs are subtracted from milk income to obtain the net return over feed costs, a measure of farm profitability. Simulations over many years of weather provide long term evaluations. Output information includes field curing times, amounts and quality of feeds produced, amounts and nutrient contents of manure handled, production costs and the net return to the producer. All simulation results are provided for each year of weather to provide both a mean and a distribution from best to worst weather conditions.

Both the mean and variance of simulation results are used to compare forage systems. The mean of production costs and/or the net return over production costs provides estimates of the long term costs and profitability of one technology versus another. The variance is an indication of the risk of using a given system. With this variance, alternative technologies can be compared by their ability to reduce the producers risk in dealing with weather. This type of information helps direct research toward the more promising technology and aids forage producers in adopting the best technology for their farms.

Figure 2. Swath manipulation by tedding may cost more than the benefit received.

DAFOSYM is a FORTRAN program that is compiled for execution on computers using a DOS operating system. The latest version includes a package of programs for setting input parameters and viewing simulation results. Overlaying menu and spreadsheet formats are used to view and edit parameters. A plotting package provides bar graphs, pie charts and other plots of output for

Figure 3. A forage mat system can return up to $4 for each dollar spent on increased machinery, fuel and labor costs.

Figure 4. Storing large round bales outdoors is sometimes more economical than shed storage.

rapid evaluation and comparison of simulated systems. The model has been distributed to forage researchers, extension personnel and a few producers to determine its potential as a teaching and management aid. A version of DAFOSYM that runs in a Microsoft Windows environment is under development. This new version allows greater maneuverability among portions of DAFOSYM, better linking with other programs, and expansion of DAFOSYM to include more technologies.

Technologies Evaluated

A wide variety of technologies have been evaluated to determine their benefit to dairy farmers. Among the first technologies studied were those which improved the field wilting or drying of alfalfa. Chemical conditioning was found to improve hay yield and quality with the potential of providing a small economic gain. Swath manipulation treatments such as tedding were found to increase harvest costs more than the benefit received through faster drying (Figure 2). A new technology under development by the USDFRC called maceration and mat drying was found to produce large quantities of very high quality forage that could reduce supplemental feed purchases, improve milk production and thus provide a substantial economic gain to the producer (Figure 3).

DAFOSYM has also been used to compare silage systems. Direct-cut harvest of high moisture alfalfa silage was compared to traditional wilted silage systems and found to have no economic value for U.S. dairy farms. Even if a treatment such as formic acid were applied at no cost to improve fermentation, the direct-cut system would cost more than the wilted silage system due to high effluent losses and the higher cost from handling and feeding greater quantities of material. When comparing silage systems using various types of silos, top-unloaded tower stave silos provided similar economic returns as bunker systems, but bottom unloaded sealed silo systems were more costly than the benefit received. Unloading bunker silos with a device that maintains a

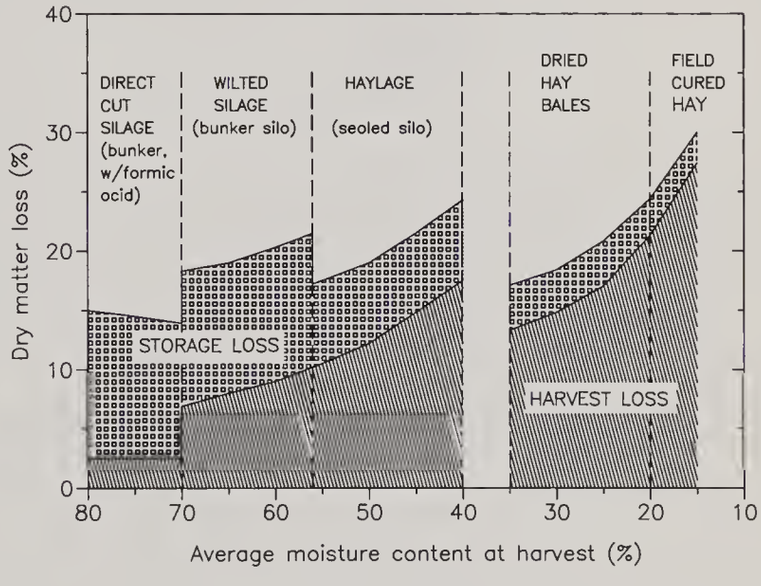


Figure 5. DAFOSYM provides an accurate evaluation of forage losses and their economic value.

smooth face can reduce silage losses, but for well managed silos the small benefit received does not appear to justify the additional equipment and labor costs required.

Other uses of DAFOSYM have included an assessment of the value of hay preservative treatments, a comparison of hay storage methods (Figure 4) and an analysis of forage system losses (Figure 5). The latest version of the model provides a tool for evaluating and comparing alternative manure systems for dairy farms and their interaction with feed production. Manure systems using long-term storage with spreading, injection or irrigation have greater direct costs to the farmer than the daily haul system commonly used in the upper midwest (Figure 6).

Figure 6. If long-term manure storage systems are required to protect the environment, the annual net cost of manure handling will increase up to $65/cow.

DAFOSYM's Future

DAFOSYM is an evolving model. Submodels of tillage and planting were recently added. This expanded model is being used to evaluate and compare the economics and environmental impact of various tillage systems and their interaction with manure handling and feed production on dairy farms. Work is underway to add grass and small grains as crop options. The emphasis of the modeling work for the next couple years will be to study how different cropping systems and feed supplementation strategies can be used to reduce nutrient loss to the environment while maintaining or improving dairy farm profitability (Figure 7). A grazing option is also being added to help determine the role of grazing and the most economical grazing systems for dairy farms in the northern U.S.

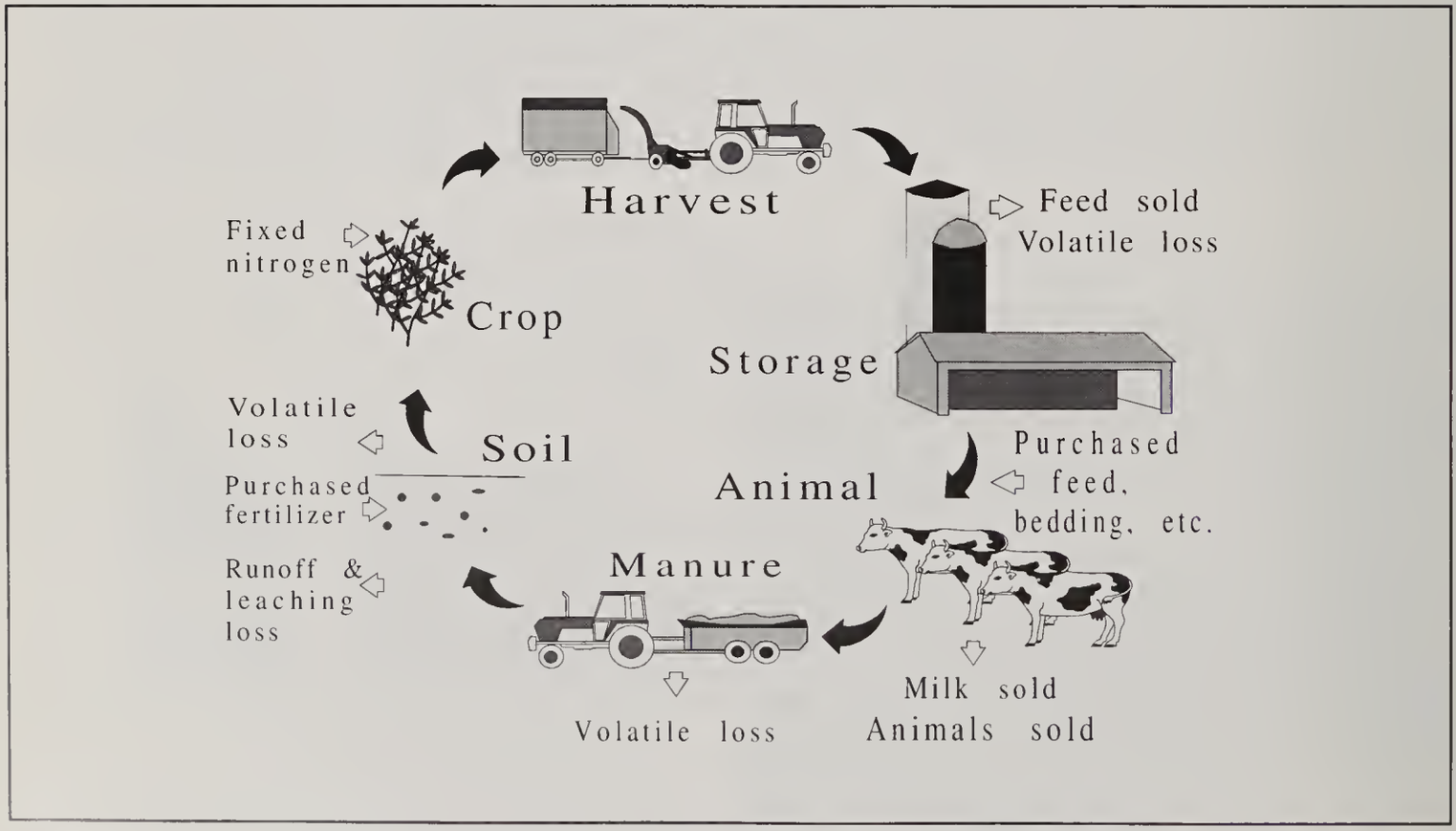


Figure 7. DAFOSYM is now being used to find the best cropping systems and feeding strategies to minimize nutrient loss from the dairy farm to the environment.

Better Red Clover Cultivars Through Selection for Persistence and Root Rot Resistance

R.R. Smith, B.C. Venuto, and C.R. Grau

Introduction

Red clover is one of the leading forage legumes in the U.S., Canada, and northern and eastern Europe. In the U.S. it has long been considered to be a short-lived perennial. Environmental and biotic stresses and root rot diseases contribute to the lack of persistence of the species. Of the root rot causing pathogens of red clover, organisms of the *Fusarium* species have been isolated most consistently from diseased roots. Therefore, resistance to these pathogens should lead to improved persistence and yield. Selection for plant longevity in older stands of red clover and the incorporation of genes for resistance to root rot pathogens are the most practical methods of improving persistence of red clover. Cultivars developed in this manner during the last two decades have excellent disease resistance, yield well, and are more persistent than cultivars developed prior to the 1970's and the so called "common" strains currently on the market. The object of this research was to evaluate red clover cultivars, "common" strains, and improved germplasm for forage yield and persistence and for their reaction to foliar and root rot diseases.

Materials and Methods

Longevity and *Fusarium* Resistance Study

Red clover germplasm developed in Wisconsin over the past four decades and a composite of the 1940's Wisconsin Common were included in this study. The cultivar, Lakeland, represented the germplasm developed in the 1950's, Arlington the 1960's, Marathon the 1970's, and population C11 the 1980's. For *F. oxysporum* evaluation, roots of 6-wk-old seedlings are soaked for 20 min in a potato dextrose broth of *F. oxysporum* microconidia and then transplanted to sterile media. Seedlings are incubated for 8 wk and then evaluated for disease severity according to a disease severity index (DSI) where 1 = a clean, white root with no visible sign of vascular browning to a 5 = death. A mixture of three *F. oxysporum* isolates retrieved from red clover plants grown in Ashland, WI, in 1988 was used. Data on *F. oxysporum* response are the means of three separate evaluations and agronomic data are the means of two field tests conducted on the Arlington Research Station, Arlington, WI.

Common Strains vs. Improved Cultivar Study

Fifty-seven lots of "common" red clover were randomly chosen from samples sent to or sampled by the Seed Division of Wisconsin Department of Agriculture for the purpose of testing germination and purity. Each lot of seed was cleaned and seeded along with the cultivar Arlington, as the check cultivar, in a replicated field test on the Arlington Research Station, Arlington, WI. Data were collected on these plots over a three year period. In addition, a replicated test was established in the greenhouse to evaluate each lot of "common" seed for its reaction to the diseases, northern anthracnose (caused by *Aureobasidium caulivora* (Kirchn.) Karak.) and powdery mildew (caused by *Erysiphe polygoni* DC.).

Results and Discussion

A major emphasis of the USDA-ARS and University of Wisconsin red clover improvement project over the past five decades has been to increase persistence and forage production of the species. Routinely, surviving plants in three- and four-year-old stands are selected and incorporated into the adapted germplasm. Both forage yield and persistence have steadily improved (2.4 vs. 3.4 Tons DM a-1 and 10 vs. 82% stand, respectively) over this period (Table 1). Correspondingly, the level of resistance to *F. oxysporum* has improved (3.00 vs. 2.16 DSI) over this same period apparently as a result of natural and/or indirect selection.

Of the 57 common lots of seed, 54 yielded significantly less forage than the improved cultivar Arlington in the second year and all produced significantly less forage over the three year period (Table 2). Yield of the common lots ranged from 60 to 95% of Arlington over the three years. Ninety-five percent of the lots had poorer stands both at the end of the first and second years. Fifty-five of the 57 lots of common seed were flowering more profusely than Arlington at the end of the second year. The percent flowering plants at the end of the first year was significantly correlated with lower forage yield in the second year. Our results would confirm earlier reports that red clovers which have a high frequency of flowering plants in the fall of the year tend to be less persistent and winterhardy.

All lots of common seed tested in the greenhouse were significantly more susceptible to northern anthracnose and powdery mildew. A high negative correlation between disease reaction and forage yield suggests that the increase in disease susceptibility contributes to a decline in yield performance.

Conclusion

Since the breeding background and origin of the "common" strains of red clover are generally unknown, the purchase and use of these strains are not sound economic practices. This study underscores the importance of using named, certified red clover cultivars for establishing persistent stands of high yielding red clover forage.

Table 1. Frequency distribution and mean disease severity index $(DSI)_a$ of the response of red clover germplasm to *Fusarium oxysporum* and third year yield and percent stand at the end of three growing years in Wisconsin.

		Percent of plants with DSI of					Mean	Dm_b	%
Germplasm	Decade	1	2	3	4	5	DSI	Yield	Stand
Common	40's	21	25	22	16	16	2.81	2.4	10
Lakeland	50's	14	25	22	25	14	3.00	2.8	24
Arlington	60's	26	23	18	18	15	2.73	3.0	51
Marathon	70's	41	24	15	11	9	2.23	3.4	65
C11	80's	40	25	20	9	6	2.16	3.4	82

[a]DSI: 1 = no symptoms, resistant; 5 = dead plant, susceptible.
[b]Dry matter yield in Tons Dry Matter per Acre.

Table 2. Mean yield, percent stand, flowering and disease reaction of 57 lots of "common" red clover compared to the cultivar Arlington.

	"common" lots sign. from Arl.		Range of performance
Character[a]	No	%	in percent of Arlington
Second year yield (5.29)	54	95	57 - 104
Two year total yield (8.86)	57	100	60 - 95
Stand: fall seedling year (86%)	54	95	39 - 97
Stand: fall second year (63%)	55	96	16 - 90
Flowering: fall second year (25%)	55	96	93 - 360
Northern anthracnose (1.6)	57	100	169 - 290
Powdery mildew (1.4)	57	100	118 - 326

[a]Values in parentheses are performance for the cultivar Arlington. Yield = Tons Dry Matter per Acre; stand and flowering are in percent; northern anthracnose and powdery mildew rated on scale of 1 = no infected plants to 5 = > 90% of plants infected.

Host Specificity of *Fusarium Oxysporum* Isolates Pathogentic to Red Clover

B.C. Venuto, R.R. Smith, and C.R.Grau

Introduction

Fungal pathogens, particularly systemic root and vascular diseases caused by the species of *Fusarium*, are major components of the disease complex impacting red clover persistence. *Fusarium oxysporum* SCHLECT. is the most common, economically important *Fusarium* on forage legumes in Wisconsin. Red clover cultivars differ in reaction to this pathogen and resistance is related to the level of cultivar persistence. Design of a breeding program for enhancing resistance to *F. oxysporum* requires a basic understanding of the source of variation in host reaction. If the primary variation in reaction to the pathogen is due to genetic variation in host population resistance, then maximum resistance in the final population could be achieved by screening numerous host populations with one or a few highly virulent isolates of the pathogen. Conversely, if the primary variation in reaction of the host is due to variation in the pathogen, then selection for resistance to one or a few isolates would not result in resistance to other isolates. The objective of this research was to determine the virulence and host specificity of *F. oxysporum* isolates pathogenic to red clover.

Materials and Methods

To ascertain the host specificity of *F. oxysporum,* 6-week-old seedlings of red, white, and alsike clover, alfalfa, and birdsfoot trefoil were inoculated with a virulent isolate of the pathogen isolated from red clover. The "cut-bare-root soak" technique was used to inoculate the seedlings. After inoculation, seedlings were transplanted to soil in plastic flats and maintained in the greenhouse. Eight weeks after inoculation the reaction of the seedlings was determined by evaluating the degree of root rot in the roots on a scale of 1 = no disease, healthy root to 5 = complete rotting of the root to death of the plant. To determine the degree of variability in the pathogen, two red clover cultivars, Arlington and Chesapeake, were evaluated for their reaction to 22 *F. oxysporum* isolates derived from either diseased red clover or pea tissue or from field soil which had just been cropped with red clover, alfalfa, or peas. Inoculation and evaluation procedures for this latter test were identical to those described for the previous test.

Results and Discussion

Significant differences were observed among red clover cultivars and between red clover and other forage legumes in reaction to a single isolate of *F. oxysporum* known to be pathogenic to red clover (Table 1). Mild or essentially no reaction was observed on alfalfa, white and alsike clover, and birdsfoot trefoil. Significant differences were observed on red clover seedlings inoculated with isolates derived from either red clover tissue or from soil previously cropped to red clover (Table 2). When tested on red clover seedlings, those isolates derived from soil previously cropped with alfalfa gave consistent reactions but were as virulent as those derived from red clover sources. Similar results were observed among isolates derived from soil cropped to peas or from pea tissue.

Conclusion

It is evident that variation in response to a specific isolate exists within and between host species. Also it is evident that variation exists among isolates of *F. oxysporum*. The genetic mechanisms of resistance employed by a host may quickly select for races of *F. oxysporum* that circumvent that particular resistance mechanism. This leads to the conclusion that selection for resistance to a specific isolate in a specific host population would not result in consistent performance when the host is subjected to an environment with heterogeneous populations of the pathogen. Therefore, it would seem that the best selection strategy to develop resistance to *F. oxysporum* in red clover would be to use a mixture of isolates on separate populations followed by intercrossing of the selected populations.

Table 1. Reaction of five forage legumes to *Fusarium oxysporum* isolate ARCT 0 known to be pathogenic to red clover.

Forage legume	Test 9173	Test 9176	Mean
		-DSI*	
Red clover			
Chesapeake	2.99	3.25	3.13
WI Common	3.17	2.60	2.89
Lakeland	3.13	2.55	2.84
Arlington		2.38	
C11	2.17	2.08	2.13
Marathon	2.19	1.95	2.07
Alfalfa	1.41	2.02	1.72
Alsike clover	1.23	1.92	1.58
White clover	1.00	1.53	1.26
Birdsfoot trefoil	1.00	1.07	1.04
LSD (0.05)	0.47	0.50	

*DSI = Disease Severity Index: 1 = no symptons; 5 = root tissue completely brown/dead plant.

Table 2. Mean reaction of Arlington and Chesapeake red clover to twenty-two isolates of *Fusarium oxysporum.*

Isolate	Source (WI location - material)	Mean DSI*
ARC 070	Ashland - red clover soil	3.40
ARCS 073	Ashland - red clover soil	2.78
ARCT 216	Ashland - red clover tissue	3.20
MRCT 001	Marshfield - red clover tissue	3.18
MRCT 008A	Marshfield - red clover tissue	2.93
MRCT 005	Marshfield - red clover tissue	2.91
MRCT 007A	Marshfield - red clover tissue	2.78
MRCT 003	Marshfield - red clover tissue	2.76
MRCT 004	Marshfield - red clover tissue	2.71
MRCT 008B	Marshfield - red clover tissue	2.70
MARCT 007B	Marshfield - red clover tissue	2.69
MAS 071	Marshfield - alfalfa soil	3.29
MAS 041A	Marshfield - alfalfa soil	3.09
MAS 070	Marshfield - alflafa soil	3.07
MAS 041B	Marshfield - alflafa soil	3.04
ArPS 050B	Arlington - pea soil	3.02
ArPS 060	Arlington - pea soil	3.00
ArPS 059	Arlington - pea soil	2.84
F79**	*f. sp. pisi* race 1	2.76
F81**	*f. sp. pisi* race 2	3.07
F80**	*f. sp. pisi* race 1	2.71
F82**	*f. sp. pisi* race 2	3.11
LSD (0.05)		0.54

*DSI = Disease Severity Index: 1 = no symptons; 5 = root tissue completely brown/dead plant.
**Isolates *formae specialis pisi* races 1 and 2 are pathogenic to pea and were obtained from C.R. Grau.

Legume Establishment and Persistence and Total Forage Yield From Legumes Interseeded into Switchgrass

K.M. Blanchet, J.R. George, R.M. Gettle, D.R. Buxton, and K.J. Moore

Introduction

Low forage production during midsummer is a major limitation of many cool-season species, which ultimately limits productivity of livestock systems. Switchgrass is a perennial, warm-season grass alternative to cool-season grasses for summer pasture. Like other warm-season grasses grown in the North Central Region of the USA, switchgrass produces most of its growth during June, July, and August. It has a relatively high nitrogen requirement for high yields of high-quality forage. Forage legumes, because of nitrogen fixation and nitrogen transfer, could provide nitrogen for switchgrass if legumes establish and persist in mixed stands. Many of the yield and quality benefits of mixtures may be attributed to nitrogen transfer from legume to associated grass. Additionally, improved seasonal

distribution of forage from mixtures of species that differ in their annual growth patterns may also be beneficial.

The objectives of this study were to: (1) evaluate establishment success of no-till interseeded legumes into established switchgrass, (2) assess legume stand persistence and subsequent yield, and (3) identify legumes that are compatible with switchgrass. The legume effect was also compared with nitrogen-fertilized switchgrass.

Methods

Field studies were conducted in a 10-year-old stand of 'Cave-in-rock' switchgrass grown near Ames, IA. Eleven legume renovation treatments and four nitrogen levels were compared by seeding legumes in 1991 and 1992. The legumes were biennial 'Polara' white-flowered sweetclover and 'Madrid' yellow-flowered sweetclover; 'Norcen', an intermediate growth habit birdsfoot trefoil, and 'Fergus', a prostrate birdsfoot trefoil; 'Apollo Supreme', a fall-dormant alfalfa, and 'Alfagraze', a pasture alfalfa; mammoth red clover and 'Redland II' medium red clover; 'Emerald' crownvetch; common hairy vetch; and a 50:50 mixture of intermediate birdsfoot trefoil and medium red clover. Nitrogen levels for nonrenovated plots were 0, 60, 120, and 240 kg nitrogen ha^{-1}.

Switchgrass was clipped by a rotary mower to a 5-cm height in early spring before interseeding. Legumes were no-till interseeded in early April of both years in 15-cm rows by using a seeder equipped with double-disk openers and press wheels. An attached toolbar carried in front of the seeding unit was equipped with ripple-type coulters for cutting through switchgrass crowns and residue. Plots were defoliated with a flail harvester at a 15-cm height in early June to minimize switchgrass competition during legume establishment. The plots were harvested during the following year at a 10-cm height in June, and at a 20-cm height in July and in August.

Results and Discussion

Excellent legume establishment was observed in June, 2.5 months after interseeding, with mean legume plant density of 195 and 163 plants m^{-2} for 1991 and 1992 seedings, respectively (Table 1). Both alfalfa cultivars and hairy vetch had highest percentage establishment. Legume renovation resulted in 9% greater yields than unfertilized grass in July of the seeding year. Average legume persistence to June of the 2nd year was nearly 50% for 1991 and 30% for 1992 seedings; less in 1992 because of greater winter losses associated with ice sheeting and cold winter temperatures. Only the trefoils and trefoil-red clover mixture accounted for more than 50% of total grass-legume stem density in mid- to late-summer of the 2nd year. Second-year yields from legume renovated switchgrass were significantly higher than they were for moderate to heavy nitrogen fertilization.

Conclusion

Legumes can be successfully established into Cave-in-rock switchgrass and can be maintained into the following year. Livestock producers can improve forage yields and seasonal distribution of forage supply by renovating switchgrass pastures with cool-season legumes. No-till interseeding of cool-season legumes into established switchgrass generally resulted in excellent initial seedling densities with good persistence through the second year. Most legumes interseeded into switchgrass achieved adequate stand densities to benefit the mixed sward unless severe winter conditions reduced legume plant survival. After legume establishment, we believe that defoliation in early June is important to minimize legume competition to established switchgrass. Livestock producers probably should renovate only a portion of switchgrass pastures in a single year because of a shortfall in forage supply that will occur during legume establishment compared with that of nitrogen-fertilized grass.

Table 1. Seeding rate as pure live seed (PLS), first-year June plant density, and percentage establishment (PE) of legumes interseeded into established switchgrass in 1991 and 1992.

		1991 interseeding		1992 interseeding	
Legume treatment	Seeding rate (PLS)[a]	June plant density	PE[b]	June plant density	PE
	seeds m^{-2}	plants m^{-2}	%	plants m^{-2}	%
White sweetclover	438	181	41.3	142	32.5
Yellow sweetclover	412	243	59.0	172	41.7
Norcen trefoil	520	254	48.9	199	38.3
Fergus trefoil	561	252	44.8	227	40.4
Apollo supreme alfalfa	371	255	68.4	257	69.3
Alfagraze alfalfa	432	307	71.0	255	59.0
Mammoth red clover	344	102	29.5	91	26.4
Medium red clover	375	194	51.7	175	46.6
Crownvetch	171	89	51.8	54	31.5
Harry vetch	52	39	75.7	33	63.7
Trefoil/red clover	260	231	51.6	187	41.7
Mean		195	54.0	163	44.6
LSD (0.05)		33	12.1	43	14.9

[a]Pure live seed.

[b]Percent establishment = (legume plant density/PLS seeding rate) x 100.

Frost-Seeding Legumes into Established Switchgrass: Establishment, Persistence, and Yield

R.M. Gettle, J.R. George, K.M. Blanchet, D.R. Buxton, and K. J. Moore

Introduction

Livestock producers can improve forage yields and seasonal distribution of forage supply by renovation of switchgrass pastures with cool-season legumes. Mechanical renovation requires specialized equipment to prepare a seedbed and to plant the legumes. A simpler, readily accessible establishment method is to broadcast legume seed on the soil surface in late winter (frost seeding). Legumes have been successfully frost-seeded into cool-season grasses in the North Central Region of the USA. Soil movement by freezing and thawing and by rainfall generally provides adequate seed-soil contact. Establishment through frost seeding is most successful when the soil surface is honeycombed with ice crystals. Recommended steps for frost seeding include minimizing competition from existing sod or residue. Too much residue may suspend the seed in vegetation or may provide too much shade to small seedlings.

The objectives of this study were to evaluate establishment and persistence of six forage legumes when frost seeded into established switchgrass and to measure the effect on subsequent forage yield.

Methods

Field studies were conducted in an established stand of switchgrass grown near Ames, IA. Plant residue above a 20-cm height was removed in late fall of the year before initiation of the study. Switchgrass stubble above a 7.5-cm height was clipped and removed in late winter, leaving approximately 4.3 Mg ha^{-1} residue on the soil surface. Six legume

Table 1. Legume establishment and persistence during the seeding year and the second year for 1991 and 1992 seedings.

Seeding year	Legume	Establishment[a] Year 1 June	Persistence[b] Year 1 July	Year 1 Sept.	Year 2 June	Year 2 Sept.
		%			%	
1991	White sweetclover	15.7	96	70	43	0
	Yellow sweetclover	21.7	97	81	49	0
	Birdsfoot trefoil	22.1	104	78	67	60
	Red clover	35.9	90	69	58	56
	Alfalfa	21.2	97	64	65	51
	Red clover/trefoil	28.7	108	86	70	69
	(Red clover component)[c]	36.7	107	95	73	83
	(Birdsfoot trefoil component)[c]	21.7	111		69	60
	Mean	24.2	99	75	59	39[d]
	LSD (0.05)	9.5	25	26	21	35
1992	White sweetclover	15.9	97	49	6	0
	Yellow sweetclover	13.1	108	95	31	0
	Birdsfoot trefoil	23.9	106	81	59	45
	Red clover	32.9	119	94	36	35
	Alfalfa	39.1	104	83	14	5
	Red clover/trefoil	27.8	125	101	50	41
	(Red clover component)[c]	37.3	133	118	37	39
	(Birdsfoot trefoil component)[c]	19.4	120	88	79	50
	Mean	25.2	110	84	33	21[d]
	LSD(0.05)	3.9	24	19	14	25

[a]Establishment calculated as: (legume plant density in June of Year 1/pure live seeding rate) x 100.
[b]Persistence calculated as: (density at each time period/original June density)x 100.
[c]Red clover/trefoil components were not used to calculate the overall mean or variance.
[d]Means were calculated by using all six legume treatments, but variance was calculated by excluding the sweetclovers.

renovation treatments and four nitrogen levels were compared by seeding legumes in 1991 and 1992. The legumes were 'Polara' white-flowered sweetclover, 'Madrid' yellow-flowered sweetclover, 'Norcen' birdsfoot trefoil, 'Redland II' medium red clover, 'Apollo' alfalfa, and a 50:50 mixture of Redland II red clover and Norcen birdsfoot trefoil. Nitrogen levels were 0, 60, 120, and 240 kg nitrogen ha^{-1}. Legumes inoculated with the appropriate *Rhizobium* were mixed in sand and broadcast by hand onto frozen soil on March 19, 1991 and March 17, 1992. Plots were defoliated with a flail harvester at a 15-cm height in early June and in late July to minimize switchgrass competition during legume establishment. The plots were harvested during the following year at a 10-cm height in June, and at a 20-cm height in July and in August.

Results and Discussion

Successful legume establishment was observed, with an average of 25% of viable seed established by June (Table 1). Red clover had high establishment in both years, whereas the sweetclovers were less successful in each year. Mean legume density

was 160 (1991 seeding) and 170 (1992 seeding) plants m^{-2} in June. Legumes had excellent persistence from June into July of the seeding years, with a mean persistence of 99% and 110% for the 1991 and 1992 seedings, respectively (Table 1). High persistence likely occurred because of additional germination during late June and July. Legumes comprised a mean of 13% of total forage by July of the seeding year, and total grass-legume yield was 19% greater than non-fertilized switchgrass. Legume persistence from June into September of the seeding year declined with a mean of 75 and 84% in 1991 and 1992, respectively (Table 1).

Mean persistence to June of the 2nd year decreased to 59 and 33% for the 1991 and 1992 seedings, respectively. The large decline in 1992 resulted primarily from ice sheeting and extremely wet soil conditions in 1993. Mean legume density in June of the 2nd year was 95 and 55 plants m^{-2} for 1991 and 1992 seedings, respectively. Legumes accounted for 92 and 55% of the grass-legume yield in June with total yield that was greater than switchgrass with 240 kg nitrogen ha^{-1}.

Conclusion

Switchgrass can be successfully renovated by frost-seeding cool-season legumes. Excellent persistence of legumes occurred except when adverse weather reduced legume survival.

Water Stress Effects on Barley Cultivars Following Defoliation in the Vegetative Stage

E. El-Mzouri, and D.R. Buxton

Introduction

In low-rainfall areas, barley is often used for dual purposes in that the plants are defoliated by grazing animals while the plants are in the vegetative stage and then the crop is allowed to complete the reproductive cycle for grain production. A better understanding of physiological traits that allows some barley cultivars to possess high potential for dual-use during limited rainfall is needed as an aid in developing improved cultivars. The objective of this study was to investigate the combined effects of defoliation and water stress on plant regrowth, physiology, and forage quality of divergent barley cultivars.

Methods

Four spring barley cultivars known to differ in response to grazing under low rainfall conditions were subject to two levels of water supply (normal irrigation and stress irrigation) and to three defoliation intensities during vegetative growth [nondefoliated control, moderate defoliation (10-cm cutting height), and severe defoliation (5-cm cutting height)] in greenhouse and field experiments. In the greenhouse at Ames, IA, the amount of water applied to the stressed plants was half of that applied to the fully irrigated plants. The cultivars were planted in the field at Settat, Morocco on Oct. 27, 1992 and harvested in March 1993. These plants were grown under natural rainfall and supplemental irrigation. The total amount of water received by the nonstressed plants was 297 mm and that by the stressed plants was 257 mm.

Results and Discussion

In the greenhouse, water stress reduced plant growth by 11%, grain yield by 45%, photosynthesis of leaves by 50%, plant water potential and solute potential, and delayed anthesis. There were no significant interactions between water stress and defoliation for forage quality related characteristics. Rate of decrease in crude protein concentration with advancing maturity was reduced by water stress. The rate was 3.2 g kg^{-1} day^{-1} for fully irrigated plants and 2.1 g kg^{-1} day^{-1} for water-stressed plants. Similarly, the rate of decline in in vitro digestible dry matter (IVDDM) was reduced from

1.7 g kg^{-1} day^{-1} in fully irrigated plants to 0.8 g kg^{-1} day^{-1} in water-stressed plants. The straw of mature plants had 55% higher crude protein concentration (Table 1) and 2% higher IVDDM (Table 2) than nonstressed plants. IVDDM and crude protein concentration in straw of nondefoliated plants were positively correlated with the leaf-to-stem ratio (r=0.40 and 0.71, respectively), and turgor pressure (r=0.37 and 0.76, respectively). The high dry matter yielding cultivars had higher crude protein concentration in straw and low grain yield under water stress.

In the field, water stress reduced lodging of all cultivars except 'Asni', which was lodging resistant even under normal irrigation. Under water stress, 'Acsad 176' produced significantly higher grain and forage yields than the other cultivars, again showing its high potential for dual use in semi-arid areas.

Conclusion

Forage quality of barley straw is improved by water stress. Cultivars like Acsad 176, that have high tillering capacity, well developed-roots, high carbon exchange rates, adjust osmotically, and high yield potential, are the most suitable for dual-use under dryland farming.

Table 1. Water-deficit effects on crude protein concentration of four spring barley cultivars at different growth stages grown in the greenhouse.

Cultivar	Treatment	Tillering	Anthesis	Mature
		- - - - - - - g kg^{-1} - - - - - -		
Barlis 628	Control+	301	101	42
	Stressed++	329	146	120
Tamallalt	Control	304	134	37
	Stressed	294	150	108
Asni	Control	303	147	36
	Stressed	291	158	101
Acsad 176	Control	292	133	46
	Stressed	285	177	106
S.E.		3	5	10

Control+ = normal irrigation
Stressed++ = stressed irrigation

Table 2. Water-deficit effects on in vitro digestible dry matter concentration of four spring barley cultivars at different growth stages grown in the greenhouse.

Cultivar	Treatment	Tillering	Anthesis	Mature
		- - - - - - - g kg^{-1} - - - - - - -		
Barlis 628	Control+	777	722	621
	Stressed++	754	717	648
Tamallalt	Control	774	716	632
	Stressed	765	722	663
Asni	Control	749	706	606
	Stressed	757	717	667
Acsad 176	Control	762	725	633
	Stressed	756	723	642
S.E.		3	1	4

Control+ = normal irrigation
Stressed++ = stressed irrigation

Barley Growth, Development, Yield, and Forage Quality as Affected by Early Defoliation

E. El-Mzouri, and D.R. Buxton

Introduction

Barley is sometimes grown for dual purposes in that it is grazed during early vegetative stages and then harvested for grain production. The practice is most common in low-rainfall areas of the world. This study was conducted to identify cultivar variation in response to defoliation, to relate physiological changes after defoliation to plant regrowth and grain yield, and to identify the effects of defoliation on forage and straw quality.

Methods

Four barley cultivars, divergent in growth pattern, were subjected to three defoliation intensities [non-defoliated control, moderate defoliation (10-cm cutting height), and severe defoliation (5-cm

cutting height)] at two growth stages (early joint and late joint) in a greenhouse study at Ames, IA and a in field study at Settat, Morocco. The field experiment was planted on Oct. 27, 1992 and harvested in March 1993. The limited rainfall at Settat was supplemented with sprinkler irrigation so that total water received during the experiment was 287 mm.

Results and Discussion

The highest grain yielding cultivars had higher forage quality at anthesis than the lowest grain yielding cultivars. Cultivars varied markedly in response to defoliation. In the greenhouse, defoliation decreased subsequent vegetative production by 18 to 40%, grain yield by up to 40%, grain size, and delayed anthesis date and shortened the grain filling period (Table 1). However, in the field in Morocco, defoliation increased the yield of two cultivars because it decreased lodging. All defoliation treatments reduced subsequent tiller in the greenhouse but often increased tillering in the field. 'Acsad 176' produced higher grain yield than the other cultivars even when severely defoliated.

The cultivars differed in forage quality related traits. Crude protein concentration was highest in Acsad 176, which was 156 g kg^{-1} at anthesis and 39 g kg^{-1} in the straw of mature plants. 'Barlis 628' had 5.5% higher in vitro digestible dry matter (IVDDM) than the other cultivars. The effect of defoliation on subsequent forage quality was generally small and varied with cultivar. For example, moderate defoliation increased IVDDM of one cultivar at anthesis and straw IVDDM of another cultivar at maturity. But it also decreased IVDDM at anthesis in two of the cultivars and in the straw of two cultivars at maturity.

Conclusion

Cultivars varied greatly in response to defoliation for most traits investigated except for those related to forage quality. Defoliation during the vegetative stage had little effect on subsequent forage quality. Acsad 176 seems to be best adapted for dual purpose use of the cultivars investigated.

Table 1. Defoliation effects on leaf area, vegetative and reproductive periods, grain yield and its components of four barley cultivars grown in the greenhouse.

Cultivar	Defoliation	Vegetative period	Reproductive period	Leaf area at anthesis	Tillers per pot at harvest	Spikes per pot at harvest	Grain yield	Grain weight
		----- days -----		cm^2			g/pot	mg
Barlis 628	Control	74.0	29.5	919	42.5	36.0	17.5	50.6
	Moderate	75.0	28.5	1132	31.9	27.5	16.1	52.4
	Severe	75.0	28.5	749	24.9	18.1	10.8	50.7
	Early	74.0	29.5	1175	28.2	21.2	15.2	52.7
	Late	76.0	27.5	706	28.7	24.4	11.7	50.3
Tamallat	Control	67.5	38.5	633	59.3	42.2	16.9	48.2
	Moderate	72.8	37.0	625	42.3	34.0	14.4	45.6
	Severe	72.5	38.5	546	36.3	26.6	10.4	43.4
	Early	72.5	37.0	685	36.4	29.8	12.8	41.6
	Late	72.8	38.5	486	42.2	30.9	12.0	47.3
Asni	Control	98.5	30.0	1187	56.8	40.5	18.1	40.7
	Moderate	101.5	28.0	1146	45.2	29.8	15.1	40.7
	Severe	101.5	28.0	704	36.2	24.8	10.7	40.1
	Early	101.5	27.0	1063	37.8	26.9	14.4	40.9
	Late	101.5	29.3	787	43.5	27.8	11.4	39.7
Acsad 176	Control	67.0	39.3	1483	51.3	35.3	23.6	52.4
	Moderate	71.5	36.8	1087	33.8	25.7	19.7	53.4
	Severe	71.5	34.8	822	31.6	22.7	15.6	49.8
	Early	71.5	37.0	842	31.0	23.5	17.7	53.1
	Late	71.5	34.5	1067	34.5	24.9	17.6	50.1

Predicting First-Cut Alfalfa Yields From Preceding Winter Weather Conditions

J.C. Durling, O.B. Hesterman, and C.A. Rotz

Introduction

In areas where alfalfa stands may be injured by severe winters, the ability to estimate future alfalfa yield in the spring would be helpful to farmers for estimating their potential forage shortfalls or to decide whether to maintain or replace an injured stand. Identified causes of alfalfa winter injury include temperature fluctuations, lack of snow cover, and persistent ice sheeting. Although the effects of over-winter weather conditions are documented, few studies have attempted to quantify the impact of winter weather on yield. The overall objective of this study was to develop a model for predicting first-cut alfalfa yield based upon over-winter weather for the northcentral U.S. Specific objectives were: 1) to determine the relative importance of fall, winter, and spring weather variables on first-cut yield in the second and third years following seeding, 2) to develop a multiple regression model for predicting first-cut yield of moderately winter-hardy cultivars in the second and third years after seeding based upon over-winter weather variables, and 3) to validate the predictive model by comparing predicted with 1989 to 1993 measured yields from East Lansing.

Methods

First-cut yields in the second and third years after seeding of moderately winter-hardy cultivars, i.e., cultivars with Minnesota winter hardiness indices from 4.4 to 4.6 were obtained from Michigan State University alfalfa variety trials in East Lansing. Yield data from 1989 to 1993 were used for model validation and earlier data were used for model development. Moderately winter-hardy cultivars were included in the study for their assumed similar response to winter weather conditions and for their prevalence on Michigan farms. Second and third year after-seeding yields were used because of greater susceptibility to winter injury in these years than typically occurs in the first year. Mean yields (dry matter, t/acre) of four replications in a randomized complete block were used for model development and validation. Over-winter weather variables were summarized for discrete periods designated as prehardening, hardening, winter, and spring. These periods were delineated by cutting dates, temperature thresholds, and calendar dates.

Simple linear regression analyses were performed with each of the weather variables as the independent variable and first-cut yield as the dependent variable. This analysis was used to determine which form of a given independent variable (e.g., growing degree days at various base temperatures) was most suitable for inclusion in the multiple regression equation. Further simple regression analyses were performed using various transformations of weather variables to determine whether responses might be curvilinear. These transformations did not increase coefficients of determination between weather variables and yield; therefore, linear responses were assumed over the range of available data.

Multiple regression analysis was used to develop equations for first-cut yield in the second, third, and combined second and third years after seeding. Variables included in each equation were determined by a forward selection procedure. When a variable was significantly related to first-cut yield at more than one base temperature or snow depth, only the form of the variable with the highest r^2 in the combined second and third years after seeding was considered as a candidate, independent variable for inclusion in the multiple regression equation. Data from the second and third years after seeding were analyzed separately to develop a unique multiple regression model for each year. Moreover, a model for the combined second and third years was developed using weather data from both years and the year after seeding as independent variables.

Results and Discussion

Prediction models for first-cut alfalfa yield in the second, third, and combined second and third years after seeding are shown in Table 1. The second year model developed by forward selection included

WDD_{51} and PHPREC with negative coefficients and $SGDD_{38.5}$ and $PHGDD_{69}$ with positive coefficients. This model explained 76% of first-cut yield variability for the second production year. The third year model, including $WTC_{31,6}$ and HDAYS with negative coefficients and SGDD with a positive coefficient, explained 69% of yield variability. The combined second and third year prediction model included the same variables as the third year model. This model explained 65% of the yield variability over both years. Validation predictions averaged ±15% of measured yields in 1989 to 1992 and 33% above measured yields in 1993. In the 1993 year, conditions not included in the models (i.e., extremely wet fall and spring soil conditions) were major yield determinants.

Conclusion

Known and suspected relationships between over-winter weather variables and first-cut alfalfa yield were confirmed and quantified. Farmers should be prepared for forage yield decreases following over-winter exposure of alfalfa to greater than normal temperature fluctuations when snow cover is less than 6 inches deep.

Table 1. Models developed by forward selection to predict first-cut alfalfa yield from over-winter weather variables.

Prediction model†	R^2	F	Degrees freedom reg.	res.
$YLD_2 = 1.58 - 0.00419\ WDD_{51} + 0.00144\ SGDD_{38.5} + 0.00717\ PHGDD_{69} - 2.39\ PHPREC$	0.76	29.48	4	37
$YLD_3 = 3.17 - 0.0863\ WTC_{31,6} + 0.00119\ SGDD_{38.5} - 0.0142\ HDAYS$	0.69	24.84	3	33
$YLD_C = 3.10 - 0.100\ WTC_{31,6} + 0.00122\ SGDD_{38.5} - 0.00703\ HDAYS$	0.65	45.74	3	75

†YLD_2, YLD_3 and YLD_C = dry matter t/acre in first cut of second, third or combined second and third year after seeding, respectively, WDD_{51} = winter degree days (base 51°F), $SGDD_{38.5}$ = spring growing degree days (base 38.5°F), $PHGDD_{69}$ = prehardening growing degree days (base 69°F), PHPREC = avg. daily precipitation in prehardening period, $WTC_{31,6}$ = winter temperature cycles (base 31°F for days with < 6 in. snow cover) and HDAYS = days in hardening period.
***significant at 0.001 level.

A Dynamic Model to Predict Winterkill in Alfalfa

V.R. Kanneganti, C.A. Rotz, and R.P. Walgenbach

Introduction

With good agronomic management and favorable weather conditions, alfalfa has the potential to produce high quality forage profitably for 4-5 years. However, adverse weather conditions during winter may result in accelerated plant death, reducing crop life significantly to much shorter than 5 years. The extent of plant death due to winter stress varies widely, causing large year-to-year variations in forage production and associated economics. Due to this reason, winter-kill effects can not be ignored while evaluating different management options or alternative technologies for a farm using whole-farm simulation models, such as DAFOSYM.

A dynamic model is needed in which population dynamics, dormancy and winter injury are integrated with other factors of crop production. Existing models of alfalfa lack this integration and therefore fail to predict winter kill. ALSIM, the alfalfa model used in DAFOSYM, does not simulate over-winter processes such as cold tolerance, freezing injury, dormancy and stand loss. Consequently, DAFOSYM analyses lack winter-kill effects. The objective of this study was to build a dynamic model to predict population kinetics in alfalfa as a function of cutting management, cultivar characteristics and weather.

Model Development

The processes included in the model are: germination and emergence, photosynthesis, respiration, evapotranspiration, dry matter growth and senescence, canopy development, dynamics of carbohydrate reserves, dormancy, winter acclimation, population dynamics, root growth in depth and weight, and root water uptake. Many of the growth and development processes included in the model were adapted from existing models, primarily from ALSIM. Soil processes and evapotranspiration were adapted from CERES models. The model crop produces photosynthate daily as a function of light energy intercepted by the canopy. Air temperature and water stress alter the rate of canopy photosynthesis. Daily photosynthate is partitioned among leaf, stem, root and carbohydrate reserve pools (model states).

Processes of dormancy, cold tolerance, winter injury and population dynamics were developed from concepts and experimental results published in the literature. Rate of plant death during winter is simulated as a function of cultivar characteristics (published ratings for dormancy and disease resistance), soil freezing and thawing, snow cover, ground cover (residual alfalfa biomass), soil moisture and temperature. The process of winter acclimation resulting in cold tolerance is modeled with a simulated dormancy index. Dormancy index is computed as a function of genotype characteristics, carbohydrate reserves, temperature and daylength. Cold tolerance to winter injury increases as the dormancy index increases from zero to a maximum level set by the cultivar type. The physical process of soil freezing and thawing is described as a function of soil type, ground cover, snow cover and soil moisture in the plow layer.

Model Inputs

Model requirements for user input include daily weather data (maximum and minimum air temperature, solar radiation and precipitation). Soils data required include soil type and soil texture and water holding capacity by layers down the profile (layer thickness may vary). The model simulates crop and soil processes daily and is coded in Microsoft Fortran for IBM-compatible personal computers.

Model Validation

Preliminary verification of model output indicated that it effectively simulates forage production, root growth, water use and population dynamics. Alfalfa production data from field experiments are currently being compiled for use in model validation. Data are being extracted from the literature or are being requested from researchers across the country.

Summary

A process-based alfalfa model was developed to predict population dynamics as a function of cutting management, cultivar characteristics and weather. The model uses readily available weather and soils information to predict forage production and forage quality across years, and incorporate winter-kill effects. Besides predicting forage production, the model predicts root-soil interface dynamics including root tissue turnover, and uptake of water and nutrients by roots. Therefore, this model in combination with other existing cereal models (e.g., CERES models) has the potential to analyze crop rotation strategies with respect to nutrient management and environmental impacts. The model will be available for distribution after validating its predictions using field data from across the country.

Alfalfa N Metabolism: Nitrate Supply in a Vertical Split Root System

J.M. Blumenthal, M.P. Russelle, and C.P. Vance

The use of N in intensive cropping systems has tremendously increased yields, but losses of nitrate from the soil have had deleterious effects on the quality of surface and ground water. It is generally recognized that inorganic N supply from the soil, and nitrate supply in particular, reduces symbiotic N_2 fixation by legumes like alfalfa (*Medicago sativa* L.). However, nearly all research on this topic has been based on exposure of the entire root system to nitrate. Under field conditions, it is likely that soil nitrate concentrations will vary with depth. For example, alfalfa roots may encounter high concentrations of nitrate deep in the soil profile when grown after corn (*Zea mays* L.) that received excessive N applications or when grown on contaminated sites, such as abandoned feedlots.

It is unknown how legumes like alfalfa respond to nitrate exposure of different parts of their root systems. Our objective was to measure the effect of localized (deep vs. shallow) exposure of the root system to nitrate on symbiotic N_2 fixation and N assimilation.

Materials and Methods

The experiment was conducted on vegetatively-propagated cuttings of a single 'Saranac' alfalfa plant to reduce plant-to-plant variability. Cuttings were planted in rigid acrylic tubes (5.1 cm diam. by 60 cm long) filled with silica sand and divided into two compartments by a 0.5-cm thick soft wax layer 30 cm from the upper end. The wax layer prevented movement of nutrient solution between compartments but allowed root penetration. The upper compartments were inoculated with *Rhizobium meliloti* strain 102F51. Both compartments were watered with one-half strength Hoaglund solution containing 0.5 mM nitrate.

After eight weeks of growth, plants were defoliated and treatments began two weeks later. A factorial combination of upper or lower compartment with low (0.5 mM) or high (10 mM) nitrate concentrations resulted in four treatments and another treatment received 5.25 mM nitrate in both compartments:

Treatment (Upper/Lower compartment)	Nitrate concentration (mM) Lower compartment	Upper compartment
Low/Low	0.5	0.5
Low/High	0.5	10
Medium/Medium	5.25	5.25
High/Low	10	0.5
High/High	10	10

Nitrate was labeled with ^{15}N to allow direct calculation of nitrate uptake and indirect estimation of total symbiotic N_2 fixation. A flow-through acetylene reduction technique was used to measure treatment effects on instantaneous nitrogenase activity (symbiotic N_2 fixation) on several days after treatment initiation. Treatments continued for nine days, at which time the plants were harvested. Samples were dried, weighed, ground, and analyzed for total N by Dumas combustion and ^{15}N by mass spectrometry.

The experiment was designed as a randomized complete block with eight replications for mass and N measurements and five replications for nitrogenase activity. All data were subjected to analysis of variance.

Results and Discussion

Plant growth was not affected by nitrate treatment (average plant mass was 10.9 g/plant). Nodules continued to gain in mass during the 9 days in the Low/Low treatment. Nodule weight declined as exposure to nitrate increased and was lower when nodules were exposed to nitrate than when only the non-nodulated roots were exposed.

Nitrate uptake increased with greater exposure to nitrate, but nitrate uptake was similar for the three middle treatments (Low/High, Medium/Medium, and High/Low). Instantaneous activity of the nitrogenase enzyme, the one responsible for the first step in N_2 fixation, declined within three hours of exposure to either 5.25 or 10 mM nitrate. Even though nitrate uptake was similar for the Low/High and High/Low treatments, greater declines in nitrogenase activity occurred when the nodulated portion of the roots was exposed than when deeper, non-nodulated roots were exposed. Greatest declines occurred when the entire root system was exposed to 10 mM nitrate. Specific nitrogenase activity was highest for the Low/Low treatment and lowest for the High/High treatment. The other three treatments did not differ in specific nitrogenase activity.

The cumulative amount of N derived from symbiotic N_2 fixation, as estimated by ^{15}N techniques, declined with increasing exposure to nitrate. Less N_2 fixation occurred when nodules were exposed to nitrate than when only non-nodulated roots were exposed. Plants exposed to 10 mM nitrate throughout their root system had N_2 fixation rates only 5% as large as those exposed to 0.5 mM nitrate.

Conclusion

These results show that the symbiosis between *Rhizobium* and alfalfa may be less sensitive to nitrate absorbed from the subsoil than from the topsoil. Therefore, we suggest that there are two types of response by the symbiosis to nitrate: an integrated response that is likely due to general plant N status and a localized response that occurs when the nodulated root zone is exposed to nitrate. This localized effect results from inhibited nodule growth and depressed specific activity of the nodules, whereas the general effect appears to be due primarily to reduced nodule growth. A better understanding of these two responses would help us to better predict the inputs of nitrogen by alfalfa and other legumes to our ecosystems and to manage those inputs in the most environmentally sound manner.

Subsoil Nitrate and Symbiotic N_2 Fixation of Alfalfa in the Field

M.P. Russelle, and J.M. Blumenthal

Excessive nitrogen applications have been implicated in contamination of surface water, ground water, and the atmosphere. Field studies routinely show that soil inorganic N concentrations and nitrate leaching losses are smaller under deeply rooted perennial crops, like alfalfa (*Medicago sativa* L.), than under annual crops, like corn (*Zea mays* L.). This implies that alfalfa may be able to effectively remove nitrate-N from the subsoil, but there has been no method to test this presumption directly.

Nitrogen removed in alfalfa herbage consists not only of inorganic soil N, but also atmospheric N_2 fixed by Rhizobium and N remobilized internally from crowns and roots. Herbage yields often are not affected by fertilizer N application, because N_2 fixation is normally adequate for high yields under nonfertilized conditions. Recent evidence from other experiments showed that N_2 fixation continued even with high rates of broadcast fertilizer N in the field. The effect of subsoil nitrate absorption on symbiotic N_2 fixation is unknown.

Our objectives were to: 1) develop a system to apply nitrate in the field to the lower portion of the root system independently from the upper portion, where most of the nodules are located; 2) compare removal of subsoil nitrate in herbage of two alfalfa cultivars — one that fixes N_2 and one that does not; 3) determine if topsoil water regime affects subsoil nitrate uptake; and 4) evaluate the effect of subsoil nitrate on symbiotic N_2 fixation.

Materials and Methods

The experiment was conducted at the University of Minnesota Sand Plain Research Farm, Becker, MN (45°23' N, 93°54' E) on a Hubbard (sandy, mixed Udorthentic Haploboroll), which is a loamy sand underlain by gravel at 95-110 cm. Soaker hoses were buried in parallel about 30 cm apart and 45-50 cm deep in April 1993. Fertilizer and lime were added based on topsoil tests according to University of Minnesota recommendations.

Experimental design was a strip-split plot with six replicates; subplot size was 2.4 by 1.8 m. The horizontal strip was subsoil nitrate concentration, either 0.3 mM nitrate-N in well water or 20 mM nitrate-N, both applied through the subirrigation system at least weekly or after more than 2.5 cm rainfall. Subsoil nitrate treatments began after the first harvest in 1993 and were maintained during the remainder of the 1993 growing season and throughout alfalfa growth in 1994. The perpendicular strip was topsoil irrigation schedule, either daily irrigation or conventional (1 or 2 times per week), to replace water use. The split plot was alfalfa cultivar, either Agate or Ineffective Agate (an ineffectively nodulated, near isoline of Agate), both seeded on June 14, 1993.

Subirrigation water at the high nitrate concentration was labeled with ^{15}N one time in 1993 (Sept. 25) and during five subirrigations in one regrowth period in 1994 (June 20 until July 16). Herbage was harvested on Aug. 20 and Oct. 3, 1993, and June 13, July 22, and Sept. 15, 1994. Plant N and ^{15}N concentrations were determined by Dumas combustion and mass spectrometry, respectively. All data were subjected to analysis of variance and comparison of two techniques for estimating N_2 fixation and N uptake from the soil (the isotope and difference techniques) were compared using paired t-tests.

Results and Discussion

Nitrate-N concentrations in the soil profile 1 to 2 d after subirrigation showed that uniform and small subsoil nitrate concentrations were present after subirrigation with well water (~0.3 mM nitrate-N) and that concentrations increased at all depths below about 30 cm after subirrigation with 20 mM nitrate-N solution. Thus, we were able to establish distinct subsoil nitrate concentrations in the field.

Establishment Year

Subsoil nitrate increased Ineffective Agate yield in the fall harvest by 130%, but it did not attain the yield of Agate with nitrate. As expected, there was a marked interaction between subsoil N and cultivar for yield, because subsoil nitrate increased Agate yield by only 9%. Daily topsoil irrigation increased yield an average of 7%. Ineffective Agate removed 105% more subsoil nitrate-N than Agate (31 vs. 15 kg N/ha) (Fig. 1). Exposure to high subsoil nitrate concentrations did not reduce symbiotic N fixation in Agate (average 30 kg N/ha during fall regrowth). Surface irrigation regime did not alter subsoil nitrate uptake.

First Production Year

During the second regrowth in summer, 1994, subsoil nitrate increased Ineffective Agate yield by 140%, but it did not attain the yield of Agate with nitrate. There was a marked interaction between subsoil nitrate and cultivar for yield, because subsoil nitrate did not increase effective Agate yield. Daily and conventional topsoil irrigation produced similar yields. Ineffective Agate removed 30% more subsoil nitrate than Agate (71 vs. 54 kg N/ha). Exposure to high subsoil nitrate concentrations reduced symbiotic N_2 fixation in Agate by 40% (from 78 to 46 kg N/ha) (Fig. 1). As in the establishment year, surface irrigation regime did not alter subsoil nitrate uptake.

Predicted subsoil nitrate removal was overestimated by the standard difference method in comparison to measurements by the modified isotope method (31 vs. 15 kg N/ha in fall of the establishment year and 71 vs. 54 kg N/ha in the second regrowth of 1994). Consequently, estimated symbiotic N_2 fixation averaged 61% lower using the standard difference method than the modified isotope method.

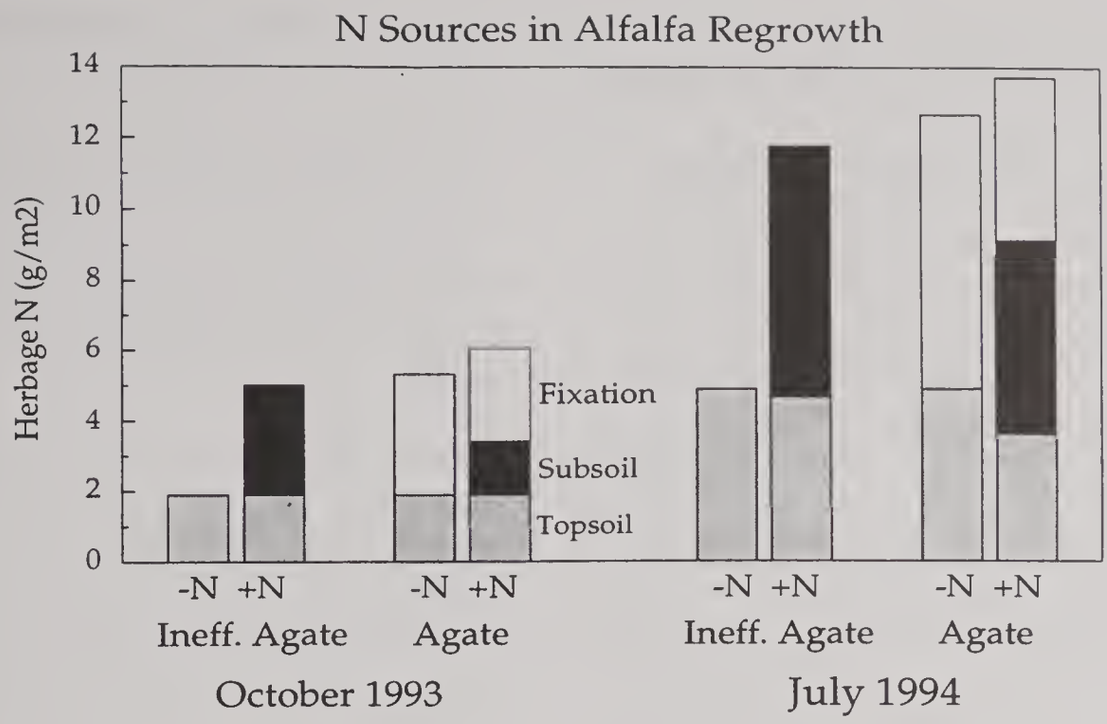


Figure 1. Sources of N in alfalfa herbage during two regrowth periods: fall regrowth during the establishment year (Oct. 1993) and the second regrowth during the first production year (July 1994). The two cultivars, Ineffective Agate (non-N_2-fixing) and normal Agate, were grown with (+N) or without (-N) 10 mM nitrate (labeled with ^{15}N) applied to the subsoil through soaker hoses at a depth of 45-50 cm.

Conclusions and Implications

We developed a system that allows nitrate application in the field to the lower portion of the root zone, independently from the upper portion. Low coefficients of variation (< 9%) demonstrate that the system produced precise estimates of herbage yield and N content. The standard alfalfa cultivar, Agate, removed less subsoil nitrate in herbage than did Ineffective Agate, despite lower yields with Ineffective Agate. Daily irrigation facilitated establishment and improved yield of alfalfa in the first year of growth but did not alter subsoil nitrate uptake. Exposure to high subsoil nitrate concentrations did not affect symbiotic N_2 fixation in the establishment year and reduced it only 40% the next.

This is the first time that direct measurements of subsoil nitrate removal have been made in the field for a N_2-fixing crop. We found substantially lower efficiency of subsoil nitrate uptake in a fixing than in a non-fixing, genetically similar alfalfa cultivar. This demonstrates that the standard difference method is invalid for estimating subsoil nitrate removal or symbiotic N_2 fixation. It raises questions about conclusions drawn in other research on subsoil nitrate removal by alfalfa. It also implies that selection of alfalfa for improved subsoil nitrate uptake may require a symbiosis more sensitive to the internal N status of the plant.

Root Growth and Distribution Are Affected by Corn-Soybean Cropping Sequence

S.E. Nickel, R.K. Crookston, and M.P. Russelle

Concern for the sustainability of agricultural practices has led to a renewed interest in crop rotations. Corn (*Zea mays* L.) and soybean [*Glycine max* (L.) Merr.] yield better when grown in rotation than when grown in monoculture, even under high inputs. The explanation for the yield increase due to rotation is not yet known. Recent research in Minnesota concluded that part of the corn:soybean rotation effect appeared to be related to improved root function, because both water use efficiency and shoot nutrient concentrations were higher in rotation than in monoculture. In addition, other work has shown that root health appeared better in both wheat (*Triticum aestivum* L.) and corn grown in rotation than in continuous monoculture.

We hypothesized that a cropping sequence of continuous monoculture would increase the susceptibility of roots to pathogens and thus decrease root vigor and development. Alternatively, an interruption of a corn or soybean monoculture by crop rotation would improve root vigor and development. The result would be more roots produced with crop rotations than with monoculture. The objective in this research was to evaluate seasonal differences in corn and soybean root development under rotation and continuous monoculture schemes.

Materials and Methods

The study was part of a long-term rotation experiment established at the University of Minnesota Southwest Experiment Station at Lamberton, MN, on a Webster clay loam (fine-loamy, mixed, mesic Typic Haplaquoll). Only the treatments of first year (first year corn or soybean after five continuous years of the alternate crop) and fourth year (four consecutive years of the same crop, representing a continuous monoculture scheme) corn and soybean were selected. The experiment was conducted during the 1991 and 1992 growing seasons. Crop management, tillage, soil fertility and pest control were maintained according to University of Minnesota recommendations. Crop sequence was established as a randomized complete block design with four replicates. Grain yields were averaged over both years for all cropping sequences. All data were analyzed by analysis of variance.

Root development was assessed by destructive sampling with soil cores and by repeated observation through minirhizotrons. Soil cores were taken at the seedling stage in both years and at flowering in the second year. Roots were washed from the segmented cores, stained with basic fuschin, and digitized with a high resolution video scanner. Root lengths were determined from digitized images. Cellulose acetate butyrate minirhizotron observation tubes were installed within several weeks after planting at a 30 degree angle from the vertical within-the-crop rows. Images of roots along the upper side of each tube were collected at weekly intervals using a high resolution miniature video camera. Videotapes were analyzed by counting root intersections along a horizontal transect across each image. Data were summed over 12.5-cm actual depth increments.

Results and Discussion

Grain yields were higher for first year corn and soybean (8300 and 2800 kg/ha, respectively) than for monoculture (7600 and 2500 kg/ha, respectively). The seasons were unusually wet at this location in both years, and 1992 was cooler than average.

As found by many other researchers, there was no correlation between root length in soil cores and root numbers in minirhizotron images. This has been attributed to a variety of causes, including the geometry of root growth, high variability among samples, efficiency of root washing, etc. We had no reason to select or reject results of either method and thus, present both.

Our hypothesis that there would be more roots on first-year corn than continuous corn was generally supported. Minirhizotron monitoring suggested that corn grown in rotation had greater root length density than continuous corn, with the exception of the top 12.5 cm, where the treatments were reversed. Soil cores confirmed more seedling roots in the top 12.5 cm on continuous corn (0.66 cm/cm^3) than on rotated corn (0.54 cm/cm^3). Our results confirm those of others who also found more roots and root activity in the topsoil under continuous corn cropping than under rotations. Our observations similarly support others who found that freshly incorporated corn residues reduced early growth of both corn roots and herbage. In the absence of such inhibition, we found that corn roots proliferated at depths of 37.5 to 50 cm in 1991 and 25 to 37.5 cm in 1992.

In contrast, our hypothesis that there would be more roots on first-year soybean than continuous soybean was generally not supported. Minirhizotron monitoring indicated that continuous soybean had as many or more roots than rotated soybean in both years, with the exception of depths 37.5 to 50 cm at flowering in 1992. Root length density data obtained from soil cores also suggested that rotated soybean had more roots at depths of 36 and 48 cm at flowering. It could be that decomposing corn residues also affect soybean root growth.

Conclusion

Growth and distribution of both corn and soybean roots were affected by cropping sequence. Fewer roots in continuous corn and rotated soybean may have been the result of autotoxins from decomposing roots of the previous corn crop. We do not have an explanation for the higher soybean yield, but poorer root growth, in rotation than in monoculture.

Draft of Major Tillage and Seeding Equipment

T.M. Harrigan, and C.A. Rotz

Introduction

Farm managers, consultants and others working with machinery management use draft information to match tractors with implements and to estimate fuel requirements. For forty years, The American Society of Agricultural Engineers has published draft and power data for agricultural equipment as a standard for use by machinery managers. Such information is required for incorporating submodels of tillage and planting in our DAFOSYM model. Many changes in tillage and planting have occurred in recent years. Tools that allow a range of control over the amount of crop residue left on the soil surface and combination tools that combine multiple tillage operations are now in common use. With over fifteen years since the last revision of the ASAE draft data, a thorough review of available information and revision of this standard data is required. Objectives of this study were to develop: 1) a model that predicts average drafts for major tillage and planting operations, and 2) develop a reference table of machine and soil specific draft parameters.

Methods

A general model was developed to predict the draft of tillage and planting equipment from data obtained through a thorough literature review. The draft force was a function of soil type, implement width, operating speed, operating depth, and geometry of the tillage tool. Soil was categorized as fine, medium or coarse. The major effort in model development was determining the machine and soil specific parameters. In some cases, machine parameters were published for specific conditions and, based on a comparison of data across soil textural groups, the parameters were extrapolated to a wider range of conditions. If parameter estimates were not previously published, the draft/speed relationship was estimated from

Table 1. Draft parameters for major tillage implements on fine, medium and coarse textured soils.

Implement	Soil texture	SI units	Parameters, SI A	B	C	English units	Parameters, English A	B	C	Range* ±%
Subsoiler	fine	N/t/cm	294	—	2.35	lbf/t/in	167	—	3.52	60
12" winged point	medium	N/t/cm	221	—	1.77	lbf/t/in	126	—	2.65	50
	coarse	N/t/cm	133	—	1.05	lbf/t/in	76	—	1.57	40
Manure Injector	fine	N/t/cm	294	—	2.35	lbf/t/in	167	—	3.52	60
12" winged point	medium	N/t/cm	221	—	1.77	lbf/t/in	126	—	2.65	50
	coarse	N/t/cm	133	—	1.05	lbf/t/in	76	—	1.57	40
Moldboard Plow	fine	N/cm²	6.5	—	0.051	lbf/in²	9.4	—	0.191	35
	medium	N/cm²	4.2	—	0.037	lbf/in²	6.1	—	0.139	45
	coarse	N/cm²	2.7	—	0.024	lbf/in²	3.9	—	0.090	50
Chisel Plow	fine	N/t/cm	91	5.39	—	lbf/t/in	52	4.94	—	60
2" straight point	medium	N/t/cm	77	4.56	—	lbf/t/in	44	4.18	—	50
	coarse	N/t/cm	59	3.47	—	lbf/t/in	33	3.18	—	40
3" twisted shovel	fine	N/t/cm	107	6.34	—	lbf/t/in	61	5.81	—	60
or 12 to 16" sweep	medium	N/t/cm	91	5.37	—	lbf/t/in	52	4.92	—	50
	coarse	N/t/cm	69	4.08	—	lbf/t/in	39	3.74	—	40
4" twisted shovel	fine	N/t/cm	123	7.29	—	lbf/t/in	70	6.68	—	60
	medium	N/t/cm	105	6.18	—	lbf/t/in	60	5.66	—	50
	coarse	N/t/cm	80	4.69	—	lbf/t/in	45	4.30	—	40
Tandem Disk Harrow	fine	N/cm²	3.09	0.16	—	lbf/in²	4.5	0.37	—	40
primary tillage	medium	N/cm²	2.72	0.14	—	lbf/in²	3.9	0.33	—	60
	coarse	N/cm²	2.41	0.12	—	lbf/in²	3.5	0.28	—	50
secondary tillage	fine	N/cm²	2.16	0.11	—	lbf/in²	3.1	0.26	—	20
	medium	N/cm²	1.90	0.10	—	lbf/in²	2.8	0.23	—	30
	coarse	N/cm²	1.69	0.08	—	lbf/in²	2.5	0.19	—	25
Offset Disk Harrow	fine	N/cm²	3.64	0.19	—	lbf/in²	5.3	0.44	—	40
primary tillage	medium	N/cm²	3.20	0.16	—	lbf/in²	4.6	0.37	—	60
	coarse	N/cm²	2.84	0.14	—	lbf/in²	4.1	0.33	—	50
Field Cultivator	fine	N/t/cm	46.0	2.77	—	lbf/t/in	26.0	2.54	—	40
primary	medium	N/t/cm	39.0	2.35	—	lbf/t/in	22.0	2.15	—	30
	coarse	N/t/cm	30.0	1.79	—	lbf/t/in	17.0	1.64	—	20
secondary	fine	N/t/cm	32.0	1.94	—	lbf/t/in	19.0	1.78	—	30
	medium	N/t/cm	27.0	1.65	—	lbf/t/in	16.0	1.51	—	25
	coarse	N/t/cm	21.0	1.25	—	lbf/t/in	12.0	1.15	—	20
Row Crop Planter										
Conv. till	all	N/row	1,550	—	—	lbf/r	350	—	—	25
No-Till	fine	N/row	1,820	—	—	lbf/r	410	—	—	25
	medium	N/row	1,750	—	—	lbf/r	395	—	—	25
	coarse	N/row	1,670	—	—	lbf/r	375	—	—	25
Zone-Till	fine	N/row	3,400	—	—	lbf/r	765	—	—	35
	medium	N/row	3,200	—	—	lbf/r	720	—	—	35
	coarse	N/row	2,800	—	—	lbf/r	630	—	—	35
Grain Drill										
Conv. Till	all	N/row	300	—	—	lbf/r	67	—	—	25
No-Till	fine	N/row	720	—	—	lbf/r	160	—	—	35
	medium	N/row	660	—	—	lbf/r	150	—	—	35
	coarse	N/row	570	—	—	lbf/r	130	—	—	35

*Variation in average draft expected due to machine and soil effects not included in the model.

published data of similar tools. Parameters selected were not necessarily the means of available data, but values which seemed most reasonable for typical operating conditions on the three major soil classifications. Because draft varies with machine design, machine adjustment, machine age and soil conditions not modeled, a range in predicted draft values was considered.

Results and Discussion

Draft for a given implement was modeled as a simple function of soil type and the speed of operation:

$$D = \mathbf{A} + \mathbf{B}(S) + \mathbf{C}(S)^2$$

where D is draft (kN or lbf) per unit, unit width, or cross-sectional area of the tilled zone, S is field speed (km/h or mph), and **A**, **B** and **C** are machine and soil specific parameters. Although the same equation was used for all machines, only one or two of the parameters were used to describe the draft of any given machine. Machine and soil specific parameters for major operations are listed in table 1.

Conclusion

A general model with machine and soil specific parameters was developed to predict the average draft of tillage and seeding implements under general conditions. The model and parameters are presented for consideration in the revision of ASAE Standards EP496, Agricultural Machinery Management and D497 Agricultural Machinery Management Data.

Simulation of Tillage and Planting Systems

T.M. Harrigan, and C.A. Rotz

Introduction

A challenge for dairy farmers is to manage their manure handling, tillage and planting systems in a cost effective and environmentally safe manner. An analysis of the many interactions of tillage and planting operations with other operations and processes on the dairy farm requires a systems approach. For such an analysis, a model is needed which integrates the effects of weather, machinery, labor and other relevant factors on tillage, planting, harvest, manure handling, storage and feeding of the herd. A simulation model of the dairy forage system called DAFOSYM provides a basis for such a model. The objectives of this study were to: 1) develop sub-models in DAFOSYM which integrate the effects of weather, machinery, labor and other relevant factors on tillage and planting of corn and alfalfa, and 2) compare the economics and performance of conventional no till and mulch and planting systems on a representative Michigan dairy farm.

Methods

Three tillage systems were modeled and compared on a synthesized, representative dairy farm with 60 milking animals. The analysis was performed for 26 years of East Lansing, Michigan weather to obtain a long-term evaluation of systems. Conventional tillage included fall moldboard plowing of all land requiring planting. In the spring, corn ground was disked once and field cultivated once before planting. Alfalfa ground was disked twice and field cultivated twice before seeding. Mulch-tillage included primary tillage with a coulter-chisel plow in the fall and spring seedbed tillage with a combination disk/field cultivator/rolling harrow tillage tool. Land planted in corn required one pass for manure incorporation and seedbed preparation in the spring while alfalfa land was worked twice prior to seeding. A modified no-till system was used which included fall tillage with a rolling tine aerator. The aerator buried very little residue yet loosened the soil, improved water infiltration and helped alleviate shallow soil com-

paction. Specialized no-till drills were used with coulters mounted in front of the furrow openers to cut through surface residue and till a narrow band of soil. Corn was planted with zone-till planters which used a gang of three fluted coulters to till a band of soil for each seed furrow.

Residue following grain harvest was assumed to cover 65% of the soil surface. The rolling-tine aerator reduced residue cover to 60%; chisel plowing reduced cover to 35%; and all residue was buried by moldboard plowing. Residue cover was assumed to decrease by 5 percentage units over winter leaving 0%, 30% and 55% cover in the spring for conventional, mulch-till and modified no-till systems, respectively. Manure spreading and tillage were begun in the spring as soon as the soil was thawed and soil moisture was suitable. Evaporation, and thus soil moisture, was influenced by residue cover.

Results and Discussion

Compared to conventional tillage, the mulch-till and no-till systems provided more timely planting of corn on some years which increased corn production a small amount (Table 1). Machinery, fuel and labor costs were less with the use of fewer tillage operations. With no-till, a portion of the reduced costs was offset by greater chemical costs. The net return over feed and manure costs was similar between the mulch-till and no-till systems, but the net return for these systems was about $20 per cow greater than that for conventional tillage. When manure injection was used with conventional tillage, labor constraints in the spring and fall were even greater. This system further reduced corn production, increased machinery and fuel costs and reduced the net return about $40 per cow (Table 1).

Conclusion

Systems which use reduced tillage provide less demand on labor in the fall and spring allowing more timely planting of crops. The improved timeliness and reduced input costs increase dairy farm net return about $20 per cow compared to conventional tillage and planting systems.

Table 1. Annual production information, system costs and net return over feed and manure handling costs for various tillage systems on a high producing, 60-cow dairy farm.

Production or cost parameter	Unit	Conventional tillage, Injection tankers	Conventional tillage, Spreader tankers	Mulch-till, Spreader tankers	No-till, Spreader tankers
Feed production and utilization					
Preharvest alfalfa production	t DM	348	348	348	348
High quality alfalfa hay production	t DM	68	68	68	68
Low quality alfalfa hay production	t DM	17	17	17	17
Alfalfa silage production	t DM	199	199	199	199
Corn silage production	t DM	129	130	131	131
Corn grain production	t DM	122	124	126	126
Corn grain purchased (sold)	t DM	(8)	(12)	(16)	(16)
Alfalfa purchased (sold)	t DM	27	26	26	26
Soybean meal purchased	t DM	30	31	32	32
Fat purchased	t DM	2	2	2	2
Average milk production	L/cow	10,312	10,318	10,318	10,318
Manure production and utilization					
Manure, bedding and waste handled	t WM	3,720	3,716	3,710	3,710
Manure applied to alfalfa land	t WM	1,606	1,592	1,588	1,589
Manure applied to corn grain	t WM	1,391	1,400	1,403	1,403
Manure applied to corn silage	t WM	723	725	719	719
Manure nitrogen to cropland	t	8	8	8	7
Manure phosphorus to cropland	t	3	3	3	3
Manure potassium to cropland	t	10	10	10	10
Manure fertilizer value credit	$	4,200	4,170	4,170	3,960
System costs and net returns					
Machinery cost	$	34,181	32,912	32,397	31,521
Fuel and electric cost	$	3,970	3,744	3,677	3,572
Feed and manure storage cost	$	9,571	9,586	9,583	9,583
Labor cost	$	12,292	12,332	12,189	11,922
Seed, fertilizer and chemical cost	$	7,193	7,254	7,247	8,200
Corn grain drying cost	$	2,590	2,559	2,523	2,518
Land charge	$	8,000	8,000	8,000	8,000
Net feed and bedding cost	$	13,563	13,316	13,087	13,106
Total feed and manure cost	$/cow	1,523	1,495	1,478	1,474
Income from milk sales	$/cow	2,836	2,836	2,837	2,837
Net return over feed and manure cost	$/cow	1,313	1,341	1,359	1,364

Harvest Loss From Respiration and Rain Damage

C.A. Rotz

Introduction

Substantial dry matter (DM) and nutritive losses occur during forage harvest due to plant and microbial respiration and rain damage. This loss is primarily the extraction or depletion of plant nutrients, but rain may also disassociate forage material, primarily leaves. Since leaves have a higher concentration of nutrients than stem tissue, leaf loss causes a reduction in the nutrient concentration of the remaining forage. Nutrients extracted from the forage are primarily readily available carbohydrates and other soluble components. Models have been developed to predict DM and nutrient losses during forage harvest, but these models were derived for particular species and regions of the world. The objective of this work was to develop simple models applicable to a range of crop species which predict DM and principal nutrient losses caused by respiration and rain damage during forage harvest.

Methods

Loss models were developed using data and other available information drawn from a comprehensive literature review. Although these models were primarily empirical in nature, a theoretical view of the physical and biological processes involved was used to help establish the functional form and bounds of models. When enough data were available, independent data sets were used to create and validate individual models. When data were limited, models were simply created to predict the available data. In most of these cases, data were available for grass and legume crops and more than one region of the world, so the predictive models should be reasonably accurate for general use.

Results and Discussion

Respiration performs a vital role in living plants, and this process continues after the crop is mowed. Oxygen is used to convert plant carbohydrates to water, carbon dioxide and heat which leave the plant causing DM loss. Respiration rate decreases with moisture content and increases with temperature. Rewetting of a dry crop by dew or rain can reactivate enzyme activity and thus prolong respiration at a rate similar to that in non-rewetted forage at a similar moisture content. Respiration losses can be modeled as a function of the respiration rate and crop drying time. The rate is primarily a function of crop temperature and moisture content:

$$R = 0.00017\, T\, m^{2.6} \qquad [1]$$

where R is the rate of respiration loss (fraction DM/h), m is the crop moisture content (fraction wb) and T is temperature (°C). One can assume that the moisture decreases linearly from some initial moisture content to a final moisture content over the field curing period. Substituting this linear moisture relationship into equation 1 and integrating from time equal zero to the total field curing time gives:

$$L_{rs} = 0.000047\, T_a\, \frac{m_o^{3.6} - m_f^{3.6}}{m_o - m_f}\, t_{fc} \qquad [2]$$

where L_{rs} is the portion of crop DM lost by respiration (fraction), m_o is the initial crop moisture content (fraction wb), m_f is the final crop moisture content (fraction wb), T_a is the average diurnal temperature (°C) and t_{fc} is the field curing time (h).

When DM is depleted from the crop, the change in concentration of any plant constituent or nutrient

can be predicted based upon its rate of loss relative to total DM loss:

$$C_f = \frac{C_i - a L}{1 - L} \quad [3]$$

where C_f is the final nutrient concentration (fraction), C_i is the initial nutrient concentration (fraction), L is the portion of total DM depleted from crop (fraction), and a is the ratio of the loss of a given nutrient to the total loss (fraction). Since the carbohydrates lost in respiration are highly digestible, a is equal to 1 when predicting the concentration of digestible nutrients (TDN, IVDMD, etc.). To predict changes in concentration of crude protein, crude fiber, acid detergent fiber, neutral detergent fiber, lignin and many other forage plant constituents not used in plant respiration, the value of a is zero.

Losses from rain damage include leaf loss and leaching loss. Leaf loss can be predicted by:

$$L_l = 0.011 (f_l) (2 - m) \sqrt{r_n / D_s} \quad [4]$$

where L_l is the portion of crop DM lost through rain induced leaf shatter (fraction), f_l is the initial portion of crop DM that is legume leaves (fraction, 0 for grass), r_n is the amount of rainfall (mm), and D_s is the mown swath or windrow area density (kg DM/m^2). Since leaves contain a higher concentration of important nutrients for the animal, any loss of leaves results in an overall reduction in nutrient concentration. The effect of leaf loss on nutrient concentration can be predicted from the change in leaf to stem ratio and the nutrient concentrations of each.

Rain also leaches water soluble nutrients from the forage. Leaching loss is predicted by:

$$L_r = \frac{0.0061 F_c (1 - NDF) (0.9 - m) r_n}{D_s} \quad [5]$$

where L_r is the portion of crop DM lost through leaching by rain (fraction), F_c is a conditioning factor (1.0 for crushing, crimping or flail conditioning, 0.8 for no conditioning, and 3.0 for macerated and matted forage) and NDF is the neutral detergent fiber concentration in the forage (fraction). The change in concentration of nutrient constituents resulting from leached DM can be predicted by equation 3. Highly digestible DM is lost, so a is equal to 1 when predicting the concentration of digestible nutrients. For predicting fiber concentrations, a is zero since little fiber or cell wall material is lost. About 30% of the loss is crude protein (a is 0.3). Since the loss is soluble nitrogen, the concentrations of water and acid detergent insoluble nitrogen, expressed as a fraction of DM, increase similar to fiber ($a = 0$).

Conclusion

Respiration loss can be predicted from crop temperature, moisture content, and drying rate. Rain damage is predicted from the fraction of the crop that is legume leaves, crop moisture content, the type of conditioning used, the cell soluble content of the forage, swath density, and the amount of rain.

Loss Models for Forage Harvest Operations

C.A. Rotz

Introduction

Forage harvest operations cause substantial dry matter (DM) and nutritive losses. These machine-induced losses are due to the shattering of plant material and the dropping of particles during the pickup and handling of the crop. Much of the loss is leaves. Since leaves have a higher concentration of nutrients than stem tissue, leaf loss causes a reduction in the nutrient concentration of the remaining forage. Previous models used to predict these losses are specific to alfalfa or grass species. Often forage crops are mixtures of grass and legume species, so models for pure stands are not

satisfactory. As forage system models are developed for application over crops in many areas of the world, there is a need for models that are not crop specific. The objective of this work was to develop simple models applicable to a range of crop species which predict DM and principal nutrient losses for each of the major forage harvest operations.

Methods

Models were developed to estimate DM loss in mowing, swath manipulation, baling, and chopping. The general procedure was the same for the various models; however, some specific differences occurred due to the type and amount of information available. The goal was to draw together available information on harvest losses for various grasses and legumes. Models were derived which gave a reasonable prediction of the available data. Although these models were primarily empirical in nature, a theoretical view of the physical and biological processes involved was used to help establish the shape and bounds of models. When enough data were available, independent data sets were used to create and validate individual models. When data were limited, models were simply created to predict the available data. In most cases, data were available for grass and legume crops and for more than one region of the world, so the predictive models should be reasonably accurate for general use.

Mechanical losses can influence the relative amounts of leaf and stem material, particularly in legumes. Since leaves have a greater concentration of many important nutrients, a greater loss of leaves causes a change in the nutrient concentration of the remaining forage. Thus, the effect of the DM loss on forage quality is predicted with the change in leaf-to-stem ratio.

Results and Discussion

Loss from a mowing and conditioning operation can be estimated as a function of the amount of delicate (legume) leaves on the crop and crop maturity:

$$L_m = 0.006\ f_m\ (1 + 2\ f_l)\ S_d \quad [1]$$

where L_m is the portion of crop DM lost during mowing and conditioning (fraction), S_d is the crop stage of development factor (1 at early to late vegetative stage for legumes, boot stage for grass, 2 at early to mid bloom stage for legumes, heading stage for grass and 3 at full bloom stage for legumes, anthesis stage for grass) and f_m is a mower factor (1.0 for a mower with conditioning rolls and 0.5 for a mower without conditioning). For a flail mower, the loss may increase by a factor of 2.0. Losses may also double when either roll or flail type conditioning devices are adjusted for very aggressive conditioning.

Major swath manipulation operations include tedding and raking. Tedding loss is modeled as a function of crop moisture content and legume leaf portion:

$$L_t = 0.044\ (1 + 6\ f_l)\ (1 - m)^{1.5} \quad [2]$$

where L_t is the portion of crop DM lost during tedding (fraction). The portion of the crop that is legume leaves (f_l) must be set to zero for grass crops, since grass leaf blades are much less susceptible to detachment. Dry matter loss with a properly adjusted side-delivery rake can be predicted by:

$$L_{rk} = \frac{0.02\ (1 + 2\ f_l)\ (1 - m)^{1.5}}{D_s} \quad [3]$$

where L_{rk} is the portion of crop DM lost during raking (fraction).

Baler loss includes pickup and chamber losses. Pickup loss, expressed as a portion of yield, decreases as the swath density or crop moisture increases:

$$L_p = \frac{0.003}{m\ D_s} \quad [4]$$

where L_p is the portion of crop DM lost at the pickup (fraction). Chamber loss can be modeled as a function of crop moisture content and baler type:

$$L_b = 0.0025\ f_b\ m^{-1.2} \quad [5]$$

where L_b is the portion of crop DM lost from the baler chamber (fraction) and f_b is a baler factor (1.0 for small rectangular baler, day time baling; 0.5 for small rectangular baler, night baling; 0.5 for mid-size rectangular baler; 1.0 for variable chamber round baler and 2.5 for fixed chamber round baler). When baling alfalfa, chamber loss consists of about 80% leaf material. With this fraction, and the chamber loss, nutrient concentrations in the remaining crop can be determined.

Losses from a forage harvester include pickup and drift losses. Pickup loss again occurs as the crop is lifted into the machine and can be predicted with equation 4. Drift losses occur as the chopped material exits the spout of the harvester and travels toward a trailing wagon or truck. Reasonable values for drift loss can be predicted by:

$$L_c = 0.002\ m^{-1} \quad [6]$$

where L_c is the portion of crop DM lost from the chopper spout (fraction). The quality of the lost material is similar to that harvested, so the loss has little effect on the quality of the remaining forage.

Conclusions

Models were developed which predict forage DM and nutrient losses during harvest for a wide range of forage species. These models are intended for use in forage enterprise models such as DAFOSYM that are used to evaluate or compare forage systems.

Intensive Mechanical Conditioning of Forage Crops to Improve Drying Rates and Animal Utilization

R.G. Koegel, T.J. Kraus, R.J. Straub, and K.J. Shinners

Introduction

Past research has shown that intensive mechanical conditioning of forages at the time of mowing can result in both higher fiber digestibility and increases in the percentage of "bypass" protein, as well as increasing field drying rates. Important relationships between the level of conditioning and the increase in specific properties have not been developed to date, however, largely because of the lack of an objective, quantitative measure of the level of conditioning.

A surface area index (SAI), based on rate of forage moisture absorption, was developed to measure level of conditioning. While this served a need, it required specialized equipment, did not give immediate results, and did not gain general acceptance.

Several researchers have shown that leaching losses, as a result of rain, increased rapidly as the level of conditioning increased. This led to the hypothesis that some property of the leachate, prepared by a standardized method, could be useful as a measure of the level of conditioning. While it was known that both color and turbidity of the leachate changed with the level of conditioning, it was decided to try using electrical conductivity as a less complicated and more convenient measure.

Method

The following procedure was developed: (1) A 25g ± 1g (wet weight) representative sample of the forage to be evaluated was placed in a 500 ml glass jar; (2) 300 ml ± 2 ml of distilled water was added

to the jar; (3) the jar was shaken on an orbital shaker at 200 cycles per minute for 2 minutes (4); the contents of the jar were filtered through 2 layers of cheesecloth, and (5) temperature and conductivity of the liquid were measured one minute after removal from the shaker using a Cole-Parmer model 1481-60 conductivity meter. Prior to testing, the distilled water was warmed to 27°-28° C and held in a Dewar flask, so that, at the time conductivity was measured, its temperature approached 25° C. The conductivity meter was calibrated, using 700 micromho NaCl solution, just prior to starting and was recalibrated at one hour intervals thereafter.

A trial was conducted to determine whether a correlation could be established between level of conditioning, as measured by electrical conductivity, and in-situ rate of alfalfa dry matter disappearance from dacron bags in rumen fistulated cattle.

Results

Figure 1 shows typical conductivity values for leachate from alfalfa conditioned at four different levels. Crushing-impact maceration was carried out by the conditioning unit used on a forage mat machine described in earlier reports, while rotary-impact maceration was accomplished by a small unit used for severely disintegrating plant material prior to juice expression.

Figure 2 shows % dry matter disappearance for the same material placed in the rumen. The disappearance rate constants correlate well with the conductivity numbers.

Conclusions

Measurement of leachate conductivity appears to be a promising method for determining level of conditioning of forage. In-situ dry matter disappearance of alfalfa placed in the rumen appears to correlate well with leachate conductivity.

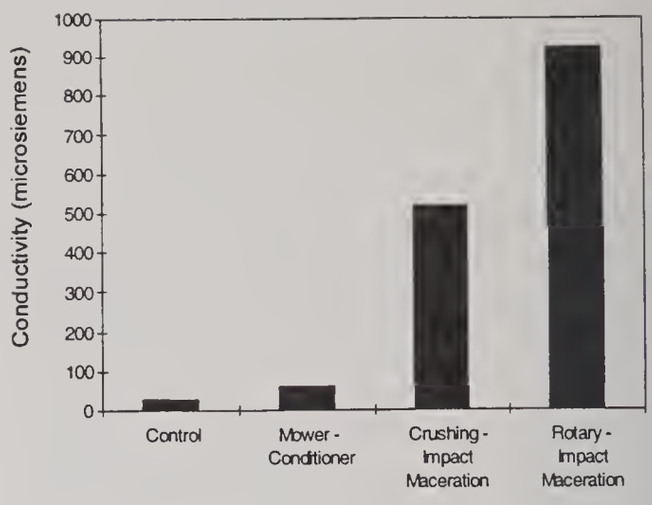


Figure 1. Conductivity of alfalfa leachate vs severity of conditioning.

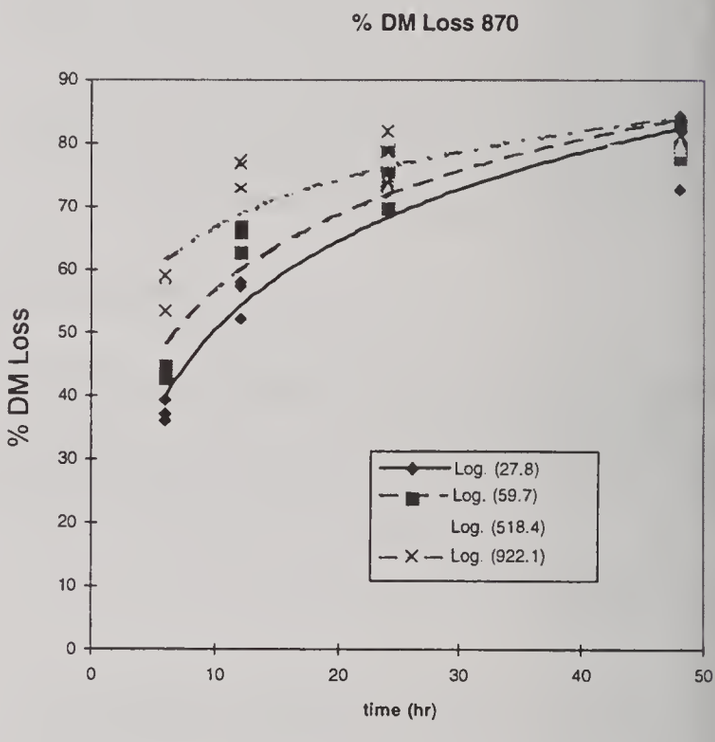


Figure 2. Dry matter disappearance of alfalfa conditioned to different levels from Dacron bags in the rumen vs time.

Fractionation of Alfalfa Juice for Value-Added Products

R.G. Koegel, and R.J. Straub

Introduction

Biotechnologists at the University of Wisconsin have created transgenic alfalfa varieties which produce industrially valuable enzymes not normally produced in alfalfa. These include alpha-amylase, widely used in converting starch to sugar, and manganese-dependent peroxidase, believed to be valuable for the bio-pulping of wood in the paper industry. Much "environmentally-friendly" processing envisaged for the future depends on an abundant and inexpensive supply of enzymes. An example of this is the hydrolysis of biomass to sugars which are fermentable to ethanol, usable as a transportation fuel.

It is planned to add the enzymes phytase and cellulase to alfalfa as soon as time and funding permits. The former allows phosphorus in the rations of monogastrics to be used more efficiently which also results in less phosphorus loading of the environment. The latter is important for the hydrolysis of ligno-cellulosics to fermentable sugars in the production of ethanol.

Target enzymes will be harvested from the juice of transgenic alfalfa. This juice contains two forms of protein: particulate (or chloroplastic) and soluble (or cytoplasmic). Since the target enzymes occur with the soluble protein fraction, maximizing the yield of this fraction is of interest. In addition, the soluble protein fraction has potential as a protein fortifier in food products. The chloroplastic fraction contains pigmenting agents, called xanthophylls, which are used in the poultry industry to color yolks and skin. The xanthophylls in the particulate protein concentrate give it a potential value of 2½ to 3 times that of its protein value alone based on the cost of currently used pigmenting materials.

Methods

The juice was clarified by the centrifugation of particulate material. Clarification was enhanced by a gentle warming and holding procedure to promote aggregation of the particulates. Aggregation temperatures ranged from 35°C to 45°C with higher temperatures giving a quicker and more complete separation. Centrifugation was carried out at 10,000 g for five minutes. Heat was added by three methods: waterbath, microwave, and ohmic heating using 60 Hertz AC electricity.

Results

The dry matter yields for the juice of the second cutting of alfalfa concentrates as a fraction of the original herbage dry matter are shown in Fig. 1. All yields decreased as maturity advanced. Similar trends were observed for first and third cuttings as well. Table 1 shows solids contents and yields for various fractions for an entire season over all treatments. More aggressive processing leads to greater yields, as has frequently been documented. However, the economics of more aggressive processing is unclear.

In late fall of both 1992 and 1993, the AC warming showed some yield advantage for the soluble protein fraction as claimed in the literature. Generally, however, neither of the three forms of warming showed a soluble protein yield advantage relative to the others.

Conclusions

Highest yields, relative to herbage dry matter, were obtained from relatively immature (bud stage to early bloom) plants. The yields from immature plant material were approximately 33% higher than the average for the entire season. Seasonal average dry matter yields as a fraction of herbage dry matter were: juice 18.3%, particulate precipitate, 7.4% and soluble coagulum, 2.7%. Soluble protein averaged about 41% of the total protein recovered from the juice.

Increasing treatment temperature and hold time at a given treatment temperature increased aggregation and subsequent precipitation of particulates in the centrifuge. Since temperature appears to be the more effective of the two, separations should be carried out at as high a temperature as is consistent with the targeted end products (e.g. without inactivating target enzymes or reduction in soluble protein yield). Since soluble protein starts to precipitate at about 50°C, this would appear to be an upper temperature limit.

Ohmic heating using AC electricity did not generally result in an increase in soluble protein yields relative to waterbath or microwave heating. An exception to this occurred in late fall of 1992 and 1993.

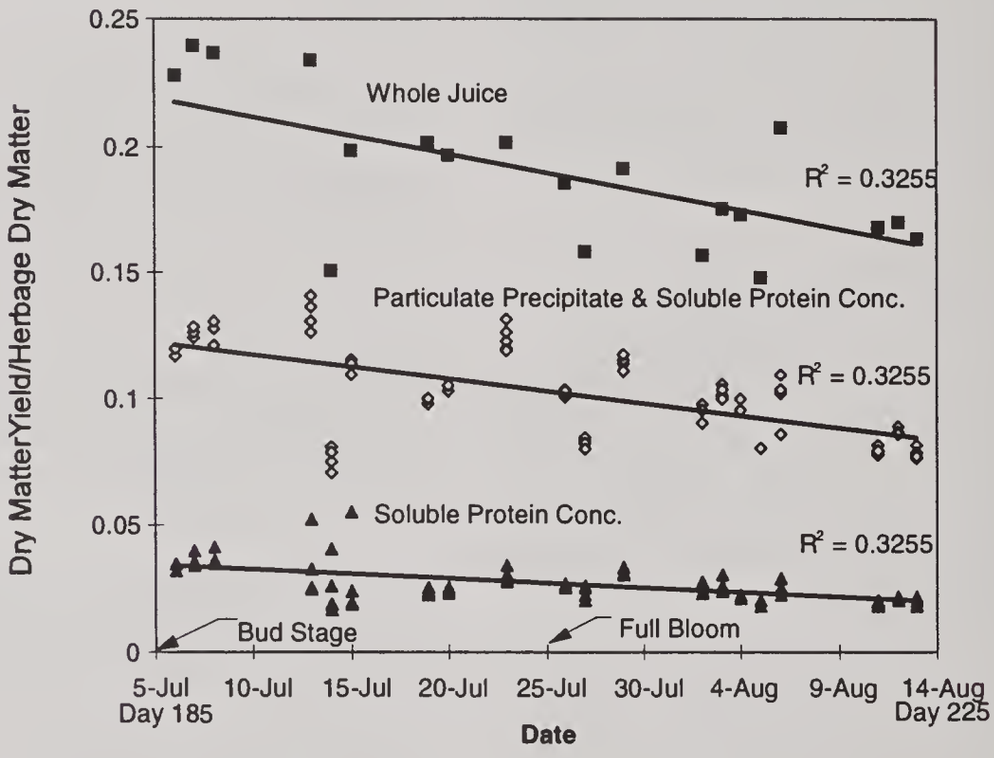


Figure 1. Yields from 2nd cutting alfalfa vs maturity; all treatments.

Table 1. Solids contents and yields, entire season; all treatments.

	Solids content			DM Yields Relative to herbage DM		
	Herbage	Juice	Fiber	Juice	Soluble protein conc.	Particulate protein conc.
Mean	.205	.097	.279	.183	.027	.075
Std. Dev.	.027	.017	.034	.025	.007	.017
Minimum	.133	.063	.204	.145	.013	.030
Maximum	.266	.150	.339	.240	.056	.123

Bioenergy From Forage Fiber

R.G. Koegel, and R.J. Straub

Introduction

Wet fractionation allows freshly cut herbage such as forage crops to be divided into a low-fiber/high protein fraction and a high-fiber fraction. While the latter fraction is an excellent feed for ruminants, it also has the potential of being a source of renewable energy. Direct combustion of forage fiber in a stationary installation is the simplest and most efficient way of recovering this energy. However, because of the great emphasis in the U.S. on transportation fuel, much interest has centered on the conversion of ligno-cellulosics, like forage fiber, to ethanol, an alternative transportation fuel.

This conversion is a two-step process in which the cellulose and hemicellulose of the fiber are first hydrolyzed by appropriate enzymes to sugars, and the sugars are fermented to ethanol by microorganisms. Highest conversion efficiencies are generally obtained when both steps are carried out concurrently in a single container. This is referred to as simultaneous saccharification and fermentation (SSF).

When ethanol is produced from corn, the ratio of ethanol energy: energy consumed in production is approximately 1:1 depending on how much energy is credited to the co-products. In the case of ethanol produced from ligno-cellulosics, this ratio is potentially around 3:1. A major cost in the production of ethanol from ligno-cellulosics is for enzymes. Therefore the price and efficacy of enzymes will have a major influence on the cost of ethanol produced from biomass such as forage fiber.

Methods

Alfalfa and grasses were fractionated on three different dates and at differing levels of severity. The least severe treatment was one pass through a rotary impact macerator followed by juice expression in a batch-type hydraulic press. The most severe treatment was two passes through the rotary impact macerator followed by two passes through a screw press with water addition between the two pressings. The resulting fiber fractions were air-dried for subsequent use. The heating value of the fiber fractions was determined by bomb calorimeter.

The dry matter disappearance of the fiber fraction versus time was determined for different levels of enzyme treatment. A mix of two enzyme products provided by the Novo Company was used.

Results

The heating value of the fiber fractions as determined by bomb calorimeter ranged from 7461-7672 BTU/lb wet basis and 8109-8362 BTU/lb dry basis. The rate and extent of hydrolysis of the fiber fraction, using commercially available enzymes, is currently being determined.

The analysis of similar double-pressed alfalfa prepared for and reported by C.D. Lu (1979) is shown in Table 1:

Table 1. Analysis of double-pressed alfalfa (Lu 1979).

Cell Wall Constituents		59.9%
Cellulose	36.1%	
Hemicellulose	15.3%	
Lignin	8.5%	
Protein		14.9%
Solubles		18.1%
Ash		7.1%
		100.0%

When compared with commonly cited values (Table 2), the double-pressed alfalfa cell walls are considerably higher in cellulose and lower in both hemicellulose and lignin. This may facilitate hydrolysis. In addition, values commonly cited for "other" constituents are about 12% while double-pressed alfalfa had about 40%, almost half of which were "soluble".

Table 2. Composition of cell wall constituents cited for "agricultural residues" and "herbaceous plants" compared with double-pressed alfalfa.

	Commonly-Cited Values (%)	Analysis (Lu 1979) (%)
Cellulose	47	60.3
Hemicellulose	35	25.5
Lignin	18	14.2
	100	100.0

Conclusions

The energy in the most fibrous 70% of a six ton dry matter per acre alfalfa crop would be approximately 69 million BTU/Acre which is the energy equivalent of approximately 500 gallons of oil.

The energy ratio (energy in fuel: energy required for production) of ethanol produced from ligno-cellulosics such as forage fiber is approximately three times that of ethanol produced from corn. A major influence on the cost of ethanol produced from ligno-cellulosics will be the cost and effectiveness of the enzymes involved in the hydrolysis of cellulose and hemicellulose to sugars.

Reference

Lu, C.D., N.A. Jorgensen, and G.P. Barrington. 1979. Wet fraction process: preservation and utilization of pressed alfalfa forage. Journal of Dairy Science 62:1399-1407.

Bunker Silo Unloaders: An Economic Analysis

R.E. Muck, and C.A. Rotz

Introduction

Front-mounted buckets on tractors, skid-steer loaders or wheel-loaders are commonly used in the U.S. to empty bunker silos. These loaders remove silage rapidly, but they produce a rough silage face. This rough face has greater porosity and greater surface area which increase silage dry matter loss. Specialized milling-type silo unloaders available in Europe produce a smoother surface. The objective of this study was to use simulation to compare the predicted losses and economics of using a bucket unloader to those of an unloader that mills the silo face under conditions representative of dairy farms in the northern U.S.

Methods

The analysis was performed using DAFOSYM, the dairy forage system model. DAFOSYM is a simulation model of alfalfa and corn growth, harvest, storage, feeding and use on dairy farms. The dairy forage system is simulated for many years of weather to determine long term performance and economics of alternative technologies and/or management strategies. By modeling several alternatives on the same representative farms, those alternatives which maximize farm production or profit can be determined.

For this study, DAFOSYM was modified to simulate the effects of unloader type on losses during silo emptying. Based on field research, the surface area for alfalfa and corn silage emptied with a bucket unloader was set at 37 and 18% higher than a smooth vertical surface, respectively. For the milling unloader, the surface area was increased 8% for both silages. Silage density at the face was also assumed to be reduced 5% by the bucket unloader. Respiration rates were modified to reflect microbial rather than plant respiration. These changes permitted DAFOSYM to reasonably predict the differences in dry matter recovery (estimated by fermentation product concentrations) measured in an earlier field study.

The two silo unloaders were compared on a representative farm for 25 years of East Lansing weather. The farm included a 100-cow herd producing 8500 L/cow/year, 85 replacement heifers, 50 ha of alfalfa and 45 ha of corn. Alfalfa cuttings 1, 3 and 4 were ensiled in a 340 t DM bunker silo; second cutting was harvested as hay. Corn silage was ensiled in another 340 t DM silo, and the remaining corn was harvested as high moisture corn and dry corn.

A 4×4 matrix of simulations was performed comparing the two unloaders. Four different wet bulk densities at emptying (560, 720, 880 and 1000 kg/m^3) and four unloading rates (5, 10, 15 and 20 cm/day) were simulated with each unloader. The different densities and unloading rates affected silo dimensions and costs. For the purposes of this analysis, both unloaders were assumed to have the same cost, fuel usage, and labor requirement as the standard bucket loader. Therefore, the economic return obtained through reduced silage losses provided the breakeven additional cost for using the milling type unloader.

Results and Discussion

Silage dry matter losses varied from 7.4 to 23.9% as influenced by unloading rate, silage density and unloader type. Losses decreased with increasing density and unloading rate. The difference in dry matter recovery between unloaders ranged from 0.5 to 3.7 percentage units in the alfalfa silo and from 0.3 to 1.5 percentage units for corn silage. The biggest differences were for the poorest management conditions, i.e. low unloading rate or low bulk density. Unloading rate had more effect than density.

Differences in average annual net return between the two unloaders are shown in figure 1. The dramatic improvement in net return with the milling-type unloader at low densities with the 5 cm/day unloading rate was caused by an interaction with milk production level. With the high losses obtained with these scenarios, particularly with the use of the bucket unloader, the forage produced could not maintain a production level of 8500 L/cow/year. With reduced losses at higher unloading rates, there was little difference in milk production between unloaders.

Considering good management (unloading rate of 10 to 15 cm/day; density of 700 to 900 kg/m^3), the difference in annual net return between unloaders ranged from $400 to $750. These increases in net return justify an additional expenditure of not more than $3000 to $6000 for equipment if fuel and labor requirements are equal between the two unloader types. However, if labor requirements are increased 10% with the milling-type unloader on the modeled farm (~5 min/day), the difference in annual net return is reduced by $284 and the breakeven price of the milling-type unloader drops $2215. A sensitivity analysis indicated that farm size and herd production level had little effect on the difference in net return between the two unloaders.

Conclusions

The DAFOSYM analysis indicates that the improved dry matter recovery obtained using a milling device to empty a bunker silo produces a $400 to $750 improvement in the annual net return on a typical 100-cow Michigan dairy farm when good silo management is used. This increase in net return justifies an additional expenditure of not more than $6000 for equipment if fuel and labor requirements are unchanged between unloader types. Because current milling-type unloaders are slower and/or more costly than bucket unloaders, farmers may not be able to justify their use at present.

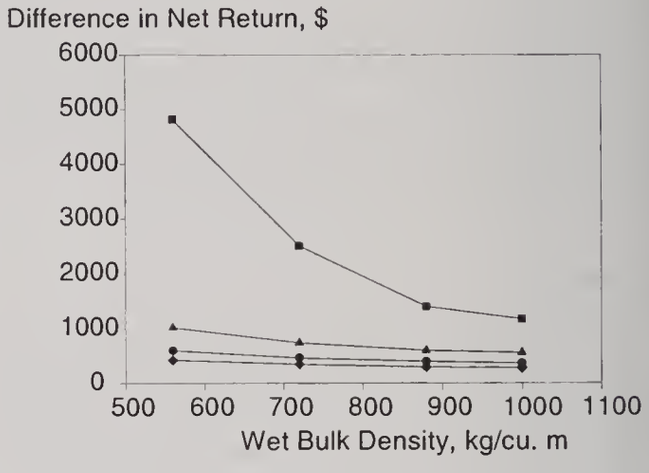


Figure 1. Predicted difference in average annual net return (milling-type minus bucket) as a function of wet bulk density and unloading rate (■ - *5*; ▲ - *10;* ● - *15 and* ◆ - *20 cm/d).*

Silage Characteristics as Affected by Length-of-Cut

R.E. Muck, K.J. Shinners, R.G. Koegel, and R.J. Straub

Introduction

Previous forage theoretical length-of-cut (TLC) recommendations were made from the standpoint of both the animal and the silo. Typical silage TLC recommendations have been historically in the 9 to 12 mm range, long enough to prevent animal health problems but short enough for the efficient mechanized filling and emptying of tower silos. The same TLC recommendations may not apply today with the movement toward bunker silos, which can handle longer TLCs, and total mixed rations, which can reduce feed particle size. Consequently, the objectives of this study were to determine the effects of a longer TLC on the rate and extent of consolidation of alfalfa under common static pressures, on compaction characteristics of alfalfa using a dynamic "drop hammer" test, and on the silage characteristics of alfalfa ensiled in a full-size bunker silo.

Methods

The consolidation and compaction tests were performed on first, second and third crop alfalfa at about 1/3 to 1/2 bloom, cut with a conventional mower-conditioner, field wilted one day and chopped with a stationary apparatus at TLCs of 9 and 25 mm. For the consolidation tests, between 9 and 12 kg wet alfalfa were placed in 29 cm diameter PVC silos. A static pressure of either 69 or 103 kPa was applied with a piston. The position of the piston with time was recorded periodically for up to 167 h. After the test, the silo contents were oven-dried to determine dry matter content and density.

A "drop hammer" apparatus was used in the compaction experiments. Approximately 2.5 kg of material was used per replicate. Approximately 1/3, by volume, was placed in the cylinder, the height of the loose material measured, and the collar on the hammer rod placed so that the hammer would be dropped 305 mm onto the forage. The weight of the hammer, rod and collar was 101 N. Twenty-five blows were applied to the forage while continuously moving the hammer around the cylinder. This procedure was repeated in two steps with the final 2/3 of the material. The final height was then measured so the final volume could be calculated. The material was then removed, weighed and oven-dried for moisture determination.

In the full-size silo experiment, first crop alfalfa at about 1/2 bloom was cut with a conventional mower-conditioner (3.7m), placed in a windrow and allowed to wilt for about 1 day. Two forage harvesters (John Deere model 3970, 12-knives, 6.5 mm TLC and New Idea model 767, 8-knives, 25 mm TLC) were used to harvest alternate windrows. Material was unloaded from front-discharge forage wagons into a 21.3 x 4.9 m concrete sidewall bunker silo. Half the silo, split along its length, was filled with each TLC treatment. The weight of each load was recorded, and three samples each were taken from each load to determine dry matter content and particle size distribution. A total of 14 and 18 loads were placed in the silo for the short and long TLC, respectively, over a two day period. After filling, a plastic cover was placed over the silage.

The following summer the silo was opened and emptied. During emptying, oxygen and temperature profiles were taken on three days at least two weeks apart. Sampling was done at 0.5, 1.2 and 1.9 m above the floor and at two sets of locations for each side. At each location, gas samples for measurement of oxygen content were collected and temperatures recorded at 12.5, 25, 50 and 100 cm from the face. Adjacent to the gas sampling locations, core samples were taken for analysis of silage density and characteristics.

Results and Discussion

In the consolidation tests at a static pressure of 69 kPa, the longer TLC reduced initial density by 7.5% and final density by 5.6%. At the higher applied pressure (103 kPa), the longer TLC reduced initial density by 12.5%, but final density was not significantly affected by TLC. These results suggest that silo capacity in tower silos would be minimally affected by the longer TLC. In the compaction tests, the longer TLC treatment reduced compacted density by 10.9%, implying a significant reduction in capacity in the initial filling of bunker silos.

When filling a full-size bunker silo with the two TLC treatments, net weight per wagon load was reduced by 33% with the longer TLC due to poor forage harvester blower performance, and 14% less weight occupied approximately the same silo volume. Final silage wet and dry bulk densities in the bunk silo were reduced 12 and 21% respectively with the longer TLC. The longer TLC silage was 2.6°C warmer on average and had higher oxygen concentrations toward the top of the silage face. Except for the reduced dry matter content of the longer TLC silage (33.6 vs. 36.9%), the two silages had similar characteristics: low aerobic microbial populations and average pHs of 4.6 to 4.7. The reduced dry matter content of the longer TLC suggests that the longer TLC side had greater dry matter losses. This should be confirmed by silage analyses which remain to be performed.

Conclusions

Chopping alfalfa with a 25 mm TLC, compared with a 9 mm TLC, had little effect on the consolidation of alfalfa at static pressures found in typical tower silo situations. However, the dynamic compaction tests and the full-size bunker silo study indicated a reduced initial and final bunker silo capacity of between 11 and 14% by using a 25 mm TLC. In the full-scale trial, there was little difference between the two TLC silages in terms of pH and aerobic microbial counts indicating similar ensiling conditions, but the reduced dry matter content and elevated temperatures on the 25 mm TLC side suggested higher dry matter losses with the longer chop length. An economic analysis of the longer TLC is needed to assess whether the reduced harvesting energy and potentially improved animal performance from the longer TLC can offset the disavantages observed during silo storage.

Effect of Modified Atmospheres on Proteolysis and Fermentation in Ensiled Alfalfa

N.F. Makoni, G.A. Broderick, and R.E. Muck

Introduction

Ensiling alfalfa results in extensive protein breakdown to NPN; this stems from lysis of cell organelles which brings together plant proteins and proteases. Despite high CP, N in alfalfa silage is poorly utilized by ruminants because of the large proportion of NPN. Preservation methods that reduce proteolysis during ensiling are essential to improving the efficiency of utilization of N in alfalfa silage. Addition of formic acid to alfalfa accelerates pH decline at ensiling and has proven effective in reducing NPN and improving animal performance. Plant respiration provides energy for maintaining the integrity of cell organelles. Modified atmospheres (MA), with lowered O_2 and increased CO_2, may extend the time during which respiration maintains organelles. These MA systems are used to extend shelf life of fruits and vegetables. The purpose of this study was to evaluate the effect on proteolysis of ensiling alfalfa under MA or N_2 gas compared to control and formic acid treated silages.

Materials and Methods

In Trial 1, fourth cutting, prebloom alfalfa was hand harvested, chopped to about 8 cm and divided into three portions for control, formic acid and MA treatments. Formic acid was applied at the rate of 6 ml/kg herbage using a misting sprayer and hand mixing. Chopped herbage was packed into 500 ml HDPE plastic bottles fitted with rubber stoppers. A total of 45 silos were filled (15 silos per treatment). Control and formic acid treatments were fitted with air traps to vent gas from the silos. Flow meters regulated O_2, CO_2, and N_2 flows into a mixing jar to give a mixture with 3% O_2 : 20% CO_2 : 77% N_2. Gas from the mixing jar passed through a pH 4.0 deionized water scrubber to ensure high relative humidity in the silos. Gas flow rate was 40 ml/min. The MA was introduced into the bottom of the forage mass and escaped out the top of the silos. Composition of gas leaving silos was monitored by gas chromatography. Silos were held at 25°C for 28 d; triplicate silos from each treatment were removed at d 2, 4, 8, 14, and 28 and frozen (-20°C) until analyzed. Only results from 28 d will be shown. In Trial 2, a field of early bloom, first-cut alfalfa was divided into two strips. One strip was cut with a mower conditioner and left in the field to wilt; the other strip was cut the following day. Both the direct cut and alfalfa wilted 1 d were chopped using a forage harvester to a theoretical length of 5 cm. Chopped alfalfa was ensiled in 2800 ml custom made PVC silos fitted with septa at the top, side and bottom. Herbage at both DM was allocated to three treatments: control, N_2 gas, and a MA gas mixture similar to Trial 1. Thirty silos were packed (5 silos per treatment). Gases were introduced into the silos as in Trial 1. Silo gas composition was

monitored by gas chromatography on samples taken through the septa. Treated silos were purged with MA and N_2 gases continuously for the first 24 h to ensure gas equilibration, then for 30 min twice daily until d 40. Silos were held at 25°C for 40 d, then were frozen (-20°C) until analyzed for chemical composition.

Results and Discussion

In Trial 1, pH of the control silage was higher than normally observed (Table 1). Buffering capacities of 550 to 600 mEquiv/kg have been reported for alfalfa; higher than normal buffering capacities were found. Very high precipitation in summer, 1993, may have accounted for the high buffering capacity of this herbage. Silage made under the MA had a pH greater than 7 (Table 1). Addition of formic acid achieved a final pH of 4.6; this reduced proteolysis. Greater NH_3 levels occurred in control and MA silages; formic acid treatment reduced but did not stop deamination (Table 1). The NH_3 was less than 10% of total N in all treatments, indicating low deamination activity. Concentrations of free AA were highest and lowest, respectively, in control and MA treated silages (Table 1). Reduced free AA in MA silage was partly a consequence of greater deamination of AA to NH_3. Extent of proteolysis, as assessed by NPN levels, was reduced 24 and 29% by formic acid and MA treatments, respectively (Table 1); magnitude of proteolysis in formic acid and MA silage was not different. The difference between total NPN and N in NH_3 plus free AA was used to estimate 'peptide N.' The MA silage contained the most peptide N. Greater amounts of peptide N may result in improved efficiency of N utilization by ruminal microbes. Senescence of green leaves is characterized by decline in chlorophyll content due to deterioration of thylakoid membranes. Rate of loss of chlorophyll pigments was reduced under MA treatment; highest concentrations of chlorophyll *a* and *b* were observed in MA silage (Table 1). The MA treatment may have reduced lysis of the chloroplasts which contain 75% of total leaf protein.

Higher DM herbage usually has lower bacterial activity, and fermentation acids have a reduced role in its preservation. In Experiment 2, alfalfa was ensiled at 20% DM (low DM, LDM) or 28% DM (high DM, HDM). Content of CP, ADF, and NDF was not influenced by any treatment (Table 2). Higher NH_3 concentrations were observed in the HDM silage (Table 2); NH_3 also was greater in HDM than LDM alfalfa ensiled under N_2 gas and MA. In all treatments, concentrations of free AA were lower in HDM (Table 2), presumably because more free AA were deaminated to NH_3. Increasing forage DM reduces plant respiration, plant enzyme activity, and microbial growth while delaying plant cell lysis. Compared to controls, NPN concentrations were reduced 7.0, 7.0, 23.4 and 25.7%, respectively, for the LDM N_2, HDM N_2, LDM MA, and HDM MA treatments. These findings indicated that anoxic conditions achieved in the silo may slightly delay proteolysis, possibly by reducing oxidation. Moreover, moderate wilting had the beneficial effect of further reducing proteolysis in MA treated silage.

Conclusions

The potential of using MA to suppress protein breakdown in ensiled alfalfa was evaluated. In two trials, alfalfa ensiled under a MA enriched with CO_2 and depleted of O_2 had proteolysis reduced to levels similar to those achieved with formic acid. The MA was more effective than ensiling under N_2 gas. These studies suggest that MA systems could be used to delay cell lysis and to reduce proteolysis in ensiled forages. However, more work is needed to investigate other treatments that could be used in conjunction with MA to achieve optimal forage nutrient preservation and silage with acceptable aerobic stability.

Table 1. Composition of alfalfa herbage and effect of formic acid[1] and MA[2] treatments on the composition and chlorophyll content of alfalfa silage3 (Trial 1).

Item	Herbage	Control	Formic Acid[1]	MA[2]	LSD
Dry matter, %	25.6	26.3	24.2	27.4	- - -
Buffering capacity, mEquiv/kg DM	697.8	825.1	850.5	910.3	- - -
Total N,3 % DM	3.4	3.5	3.6	3.6	- - -
ADF, % DM	27.7	27.9	25.9	27.6	- - -
NDF, % DM	36.2	37.1	34.5	36.2	- - -
Ash, % DM	9.8	11.0	10.8	10.4	- - -
pH	- - -	5.57[b]	4.62[c]	7.32[a]	.89
NH3 N, % TN	- - -	5.59[a]	1.41[b]	6.48[a]	1.07
Free AA N, % TN	- - -	46.9[a]	37.8[b]	21.4[c]	6.0
Peptide N, % TN	- - -	7.71[b]	6.55[b]	15.00[a]	7.17
NPN, % TN	- - -	60.2[a]	45.7[b]	42.9[b]	3.7
Chlorophyll *a*, mg/g DM	- - -	.33[b]	.47[b]	.77[a]	.08
Chlorophyll *b*, mg/g DM	- - -	.04[b]	.06[b]	.18[a]	.008

[a,b,c]Means in rows with different superscripts differ (P < .05).

[1]Formic acid was applied at 6 ml/kg fresh weight.

[2]MA = modified atmosphere (3% O2 : 20% CO2 : 77% N2).

[3]Duration of ensiling was 28 d.

[4]LSD = Least significant difference (P < .05); TN = total N.

Table 2. Chemical composition of alfalfa herbage and effect of MA[1] and N_2 gas on the composition of alfalfa ensiled[2] at two DM levels (Trial 2).

	Herbage		Control		N_2 gas		MA[1]	
Item	LDM	HDM	LDM	HDM	LDM	HDM	LDM	HDM
pH	6.19	6.52	4.29	4.76	4.30	4.86	4.50	5.10
DM3	19.9	27.7	20.4	27.5	20.3	28.0	20.0	28.0
CP, % DM	21.9	20.7	21.2	20.1	20.4	19.8	21.8	20.3
ADF, % DM	28.9	30.2	29.7	29.9	30.0	32.9	29.7	32.6
NDF, % DM	38.4	39.6	38.3	39.0	39.7	41.0	40.7	41.4
Ash, % DM	8.4	11.0	8.8	11.0	7.9	11.2	8.6	11.2
NH3 N, % TN	- - -	- - -	4.7[d]	12.0[b]	6.2[c]	14.2[a]	7.2[c]	13.6[a]
FAA N, % TN	- - -	- - -	38.5[bc]	37.4[bc]	43.0[a]	39.1[b]	36.6[c]	30.9[d]
NPN, % TN	- - -	- - -	65.9[a]	60.1[b]	61.4[b]	55.9[c]	50.5[d]	44.7[e]

[a,b,c,d,e]Means in rows with different superscripts differ (P < .05).

[1]MA = modified atmosphere (3% O2 : 15% CO2 : 82% N2).

[2]Duration of ensiling was 40 d.

[3]Lyophilized DM content.

LDM = Low DM (20%); HDM = high DM (28%); TN = total N; FAA = free AA.

Enzyme, Inoculant, and Formic Acid Effects on Silage Quality and Intake

E.M.G. Nadeau, D.R. Buxton, and J.R. Russell

Introduction

Cell-wall degrading enzymes decrease cell-wall concentration of plants and therefore can improve forage digestibility and intake. In addition to enzymes, other silage additives, such as inoculant and formic acid, as well as forage species and maturity affect silage intake. This study was conducted to compare the effects of enzymes, bacterial inoculant and formic acid on silage quality and intake of orchardgrass and alfalfa.

Materials and Methods

The study consisted of two experiments. In Experiment (Exp.) 1, the forages were harvested at three dates within spring and summer growth cycles. Herbage was treated with cellulase alone or in combination with inoculant, pectinase, or formic acid and ensiled in mini silos. Silage pH and the concentrations of ammonia-nitrogen (NH_3-N), organic acids, neutral detergent fiber (NDF), hemicellulose, cellulose, and acid detergent lignin were determined. Forages were harvested at the intermediate date in Exp. 2 and cellulase was applied alone or in combination with inoculant or formic acid. The silages were fed to lambs in a 4 x 4 Latin square design. Dry matter (DM) digestibilities were measured at ad libitum intake and at an intake of 1.8% of body weight. Also, total and digestible DM intakes as well as pH and concentrations of organic acids were measured. Forages from both experiments were wilted to 27-37% DM and then ensiled for 60 days.

Results and Discussion

Cellulase, inoculant, and formic acid decreased pH to 4.0-4.3 of the silage in both experiments. Silage pH was positively related to NH_3-N concentration in both species, except for the formic acid treated silages. Although formic acid with cellulase resulted in higher pH than inoculant treated silage, the NH3-N concentration was 21% lower for formic acid treated alfalfa silage (Fig. 1). Because of higher protein concentration, NH_3-N concentration was 31% greater in alfalfa than in orchardgrass. The silages from both experiments were generally well fermented with a high lactic acid/acetic acid ratio. Cellulase had its greatest effect on cell-wall degradation with a 30% reduction of NDF concentration in orchardgrass and a 14% reduction of NDF concentration in alfalfa.

Orchardgrass had higher in vivo DM digestibility but lower intake than alfalfa in Exp. 2. There was no species x treatment interaction. Averaged across species, formic acid+cellulase treated silage resulted in a 15% greater digestible DM intake than untreated silage with 40% of the effect due to cellulase (Table 1). Thus, cellulase improved silage DM intake by hydrolysis of cell-wall components to water-soluble carbohydrates (WSC). Formic acid probably caused a rapid pH decline, inhibited protein degradation to ammonia, and restricted fermentation of WSC.

Conclusion

Silage intake can be improved by use of cellulase in combination with inoculant or formic acid. Cellulase decreases NDF concentration, inoculant favors lactic acid production relative to acetic acid and formic acid preserves the nutrients in the silage.

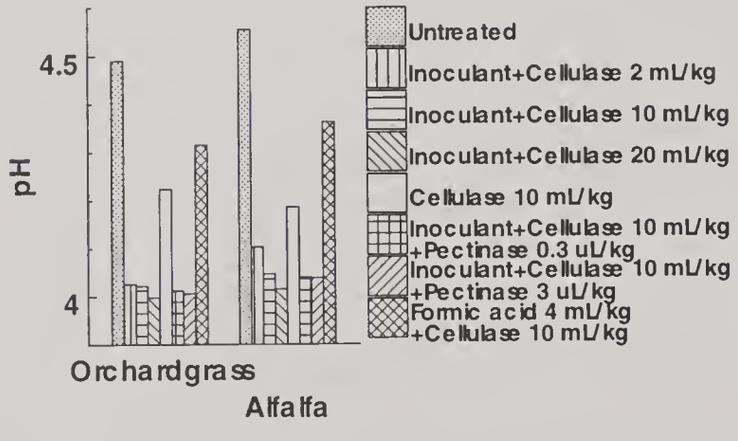


Figure 1. Effect of treatment on orchardgrass and alfalfa silages. Data are averaged across harvest dates and growth cycles. Bacterial inoculant was applied at 105 colony forming units of lactic acid bacteria/g fresh herbage. Standard error for pH = 0.02.

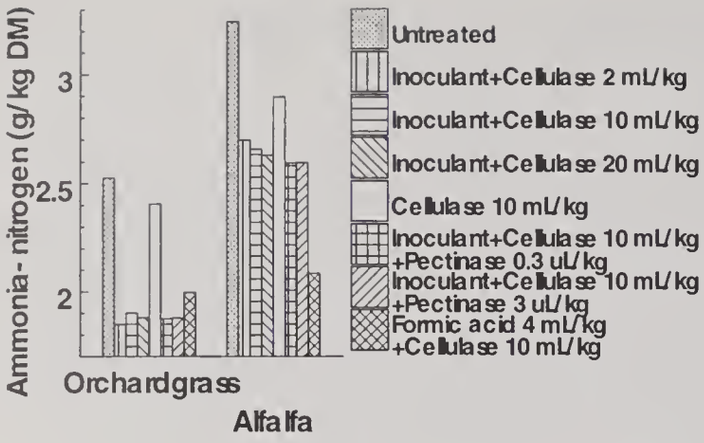


Figure 2. Effect of treatment on ammonia-nitrogen concentration of orchardgrass and alfalfa silages. Data are averaged across harvest dates and growth cycles. Bacterial inoculant was applied at 105 colony forming units of lactic acid bacteria/g fresh herbage. Standard error = 0.04.

Table 1. Effect of treatment on total dry matter (DM) intake, digestible DM intake, and DM digestibility of orchardgrass and alfalfa silages fed to lambs. Data are averaged across plant species.

Treatment	Total DM intake	Digestible DM intake	DM Digestibility[a]	DM Digestibility[b]
	- - % of body weight - -		- - - - - - - - % - - - - - - - - -	
Untreated	2.88	1.80	63.3	62.4
Cellulase 10 mL/kg	3.05	1.91	63.7	63.0
Inoculant[c]+Cellulase 10 mL/kg	3.12	1.99	64.7	63.7
Formic acid 4 mL/kg+Cellulase 10mL/kg	3.26	2.07	64.1	64.3
Significance	P = 0.08	P = 0.02	P > 0.10	P > 0.10

[a]Digestibility measured at ad libitum intake.
[b]Digestibility measured at an intake of 1.8% of body weight.
[c]Bacterial inoculant was applied at 10^5 colony forming units of lactic acid bacteria/g fresh herbage.

Cell-Wall Digestion in Situ of Enzyme+Formic Acid Treated Silage

E.M.G. Nadeau, D.R. Buxton, E. Lindgren, and P. Lingvall

Introduction

Cell-walls are only partly digested by rumen microorganisms. Cellulase degradation of cell walls during ensiling increases the easily digestible portion of the silage. As a result, ruminants can increase their forage intake and the grain portion of the diet can be decreased. The objective of this experiment was to determine the effects of cellulase in combination with formic acid on ruminal fiber digestion of orchardgrass and alfalfa silages.

Materials and Methods

This experiment was conducted at Kungsängen Research Station, Uppsala, Sweden. Freeze-dried silage samples of orchardgrass and alfalfa were weighed into bags and incubated in the rumens of two cows with two replicates in each cow. Cellulase+formic acid treated silage was compared with untreated silage. Incubation was stopped at different times up to 96 h and sample residues were then rinsed with water, dried, and weighed. Concentrations of neutral detergent fiber (NDF), hemicellulose, cellulose, and acid detergent lignin were determined. Data were fitted with a first-order, nonlinear model to estimate the potential digestion pool, digestion rate, indigestible residue (IR) concentration, and length of lag before apparent digestion.

Results and Discussion

Averaged across plant species, cellulase decreased NDF concentration by 19% during ensiling, leaving the least digestible portion of NDF for ruminal microbe digestion. Consequently, the poll of digestible NDF was 49% greater for the untreated than for the treated silage (Table 1). Cellulase + formic acid resulted, however, in 7% lower IR concentration than the control when averaged across species. There was no treatment effect on digestion rate of NDF when averaged over species, but cellulase+formic acid treated orchardgrass silage had a longer lag time than the control (Fig. 1). There were species differences; orchardgrass had 73% greater digestible NDF concentration, 32% slower digestion rate, 28% lower IR concentration and a longer lag time than alfalfa.

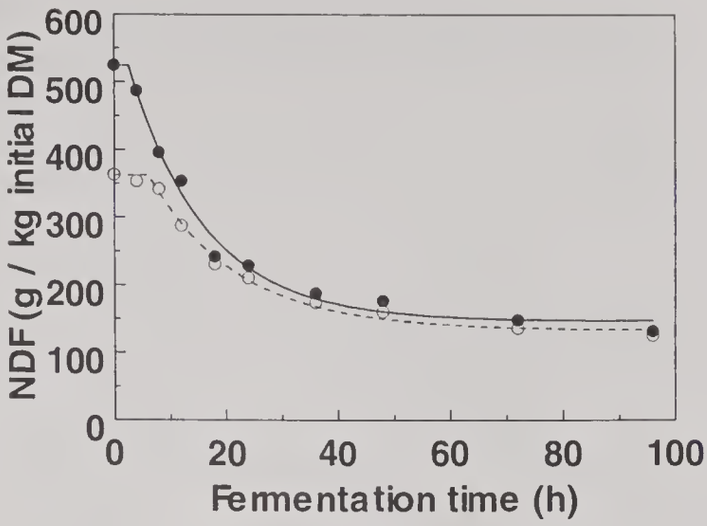


Figure 1. Neutral detergent fiber (NDF) concentration and fitted curve from nonlinear model for cellulase+formic acid treated (O) and untreated (●) orchardgrass silages.

Conclusion

As indicated by the lower IR concentration of cellulase+formic acid treated silage, more of the cell walls potentially can be utilized by ruminants. Also, cellulase combined with formic acid results in more immediate available carbohydrates for energy demanding processes, such as microbial protein synthesis in the rumen.

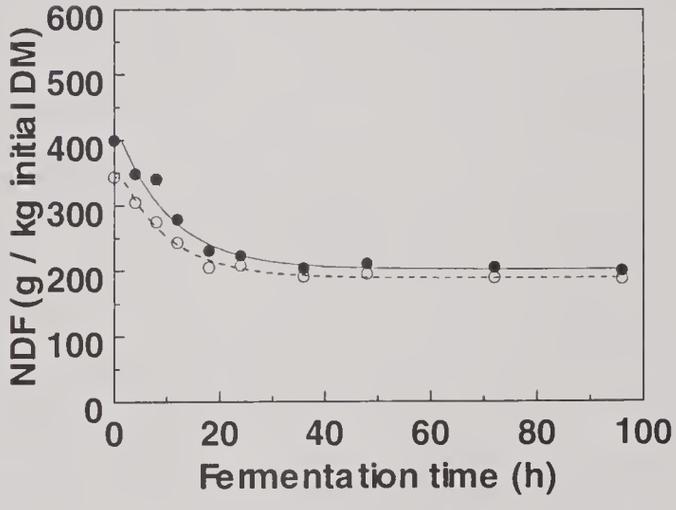


Figure 2. Neutral detergent fiber (NDF) concentration and fitted curve from nonlinear model for cellulase+formic acid treated (O) and untreated (●) alfalfa silages.

Table 1. Digestion kinetics of neutral detergent fiber of control and cellulase+formic acid treated orchardgrass and alfalfa silages.

Digestion parameter	Treatment			Species x Treatment	
Species	Control	Cellulase + Formic acid	Mean	Signifiance	$LSD_{.05}$
Digestion lag, h					
Orchardgrass	2.6	6.0	4.3***	**	1.2
Alfalfa	1.4	1.5	1.5		
Mean	2.0	3.8***			
Digestion rate, %/h					
Orchardgrass	7.4	6.4	6.9	P = 0.06	1.2
Alfalfa	9.9	10.5	10.2***		
Mean	8.7	8.5			
Potentially digestible, g/kg DM					
Orchardgrass	378	230	304***	***	7
Alfalfa	197	154	176		
Mean	287***	192			
Indigestible residue, g/kg DM					
Orchardgrass	147	133	140	NS	
Alfalfa	202	189	195***		
Mean	174***	161			

*P < .05.
**P < .01.
***P < .001.

Red Clover Inhibits Alfalfa Proteolysis

B.A. Jones, R.E. Muck, and R.D. Hatfield

Introduction

Proteolysis of ensiled alfalfa results in the degradation of 44-87% of the forage's protein during ensiling, which translates into an economic loss for ruminant producers. Interestingly, red clover, a legume of similar protein content, will have only 6-50% of its original protein degraded during ensiling. Prior research has demonstrated that the lower extent of proteolysis in red clover was not due to differences between red clover and alfalfa in total proteolytic activity or differences in pH and temperature optima and stabilities of the proteolytic activity but was due to the activity of polyphenol oxidase in red clover. The following research was undertaken to establish whether the polyphenol oxidase activity in red clover would reduce proteolysis in alfalfa silage.

Material and Methods

Three ensiling studies were performed treating alfalfa herbage with red clover herbage or extract. All legumes were harvested at early bud, wilted in a greenhouse until about 35% DM, chopped by hand (1.2 cm length), inoculated with lactic acid bacteria (10^3 live bacteria per gram herbage) and treated. Treatments were: 1) Alf, alfalfa treated with buffer; 2) RCl, red clover herbage treated with buffer; 3) Alf+RCl, alfalfa and red clover mixed 50:50 and treated with buffer; 4) Alf+RCl7, alfalfa and red clover herbage mixed 7:1 and treated with buffer; 5) Alf+Extr, alfalfa herbage treated with red clover extract (buffer extract prepared from red clover leaves); and 6) Alf+Auto, alfalfa herbage treated with autoclaved red clover extract. Final dry matter content of the treated herbage ranged between 17 to 25% depending on the study, but the dry matter content of the treated hebage within a study only varied 3% units. In studies B and C, half of the chopped herbage was macerated. Treated forages, both macerated and chopped, were ensiled into laboratory silos, incubated for three days and frozen until analysis. Fresh herbage and silages were analyzed for dry matter (DM), pH, free amino acid nitrogen (FAA-N), ammonia nitrogen (NH_3-N) and

nonprotein nitrogen (NPN), whereas fresh herbage was also analyzed for total nitrogen.

Results and Discussion

Crude protein of the alfalfa and red clover silages were 25.7 and 23.1, 24.1 and 22.0, and 27.7 and 26.7 % for studies A, B and C, respectively. Table 1 displays the pH and NPN composition of the treated silages. The pH of the silages did not decline sufficiently to inhibit proteolysis of two of the three studies. After 3 d of ensiling, when essentially all proteolysis has occurred, red clover silage was at least 40% lower in NPN than alfalfa silage. Treatment of the alfalfa herbage with either red clover herbage or extract reduced proteolysis. However, the reduction in alfalfa proteolysis was the greatest and most consistent across all studies when the treated forages were macerated prior to ensiling. Maceration of alfalfa and red clover herbage together (1:1) resulted in reduction of alfalfa proteolysis by 20-21%. A higher ratio of alfalfa to red clover herbage (7:1, treatment Alf+RCl7) resulted in a lower reduction in proteolysis (13%). This higher ratio of alfalfa:red clover herbage is similar to the amount of red clover that was used to prepare the red clover extract applied to alfalfa herbage (treatment Alf+Extr). For this reason, perhaps, a similar reduction in alfalfa proteolysis was observed in treatments Alf+RCl7 and Alf+Extr (13 and 14-17% reduction, respectively). Interestingly, the autoclaved red clover treatment did not behave consistently across all three studies. In studies A and B, the autoclaved treatment was not significantly different from alfalfa ($P > 0.08$); yet in field study C the autoclaved treatment was significantly lower than alfalfa ($P < 0.05$) and behaved the same as the red clover extract and herbage treatments ($P > 0.05$). The reason for this discrepancy is unknown but could be related to the stability and amount of the polyphenol oxidase generated quinones in the extract. These quinones generated by the enzyme polyphenol oxidase are part of the browning (bruising) reaction of plant tissue. Red clover extract in field study C had browned to a much greater extent prior to autoclaving than red clover extract in the other two studies. It is possible that quinones were sufficiently present and remained stable during autoclaving to bind to alfalfa proteins and proteases and inhibit proteolysis. This hypothesis will be investigated in future studies.

Conclusion

Polyphenol oxidase in red clover tissue can inhibit proteolysis in ensiled alfalfa. Substantial inhibition occurs only when there is adequate contact of the alfalfa tissue with the red clover treatment. Maceration of the alfalfa after red clover addition is recommended to ensure maximum treatment effectiveness.

Table 1. Effect of red clover treatments on proteolysis of alfalfa ensiled for 3 days, reported as the trichloroacetic acid soluble nonprotein nitrogen (NPN, g N/g total N).

	Physical	Study A		Study B		Study C	
Treatments	Form[1]	pH	NPN	pH	NPN	pH	NPN
Alfalfa	C	5.89	0.799	6.54	0.490	4.85	0.479
	M	——	——	6.09	0.480	4.37	0.418
Red Clover	C	5.24	0.460	——	——	4.77	0.229
	M	——	——	——	——	4.64	0.257
Alf+RCl	C	5.38	0.530	5.34	0.505	4.85	0.359
	M	——	——	5.34	0.377	4.63	0.336
Alf+RCl7	C	5.79	0.652	6.25	0.480	4.84	0.441
	M	——	——	5.82	0.416	4.47	0.365
Alf+Extr	C	6.21	0.676	6.19	0.423	4.78	0.463
	M	——	——	5.91	0.400	4.38	0.359
Alf+Auto.	C	6.64	0.782	6.38	0.502	4.82	0.407
	M	——	——	5.83	0.476	4.37	0.366

[1]Physical form of the herbage at the time of ensiling. C = chopped; M = macerated

Active Incorporation of Ferulate Polysaccharide Esters Into Lignins

J. Ralph, J.H. Grabber, and R.D. Hatfield

Introduction

Ferulates are implicated in cross-linking grass cell wall polysaccharides with lignin (see 1993 Research Summaries, p. 38). Ferulate-polysaccharide esters 'attach' to lignins during wall development. There are two possible mechanisms for this attachment, one 'active' and the other 'passive.' Although the passive mechanism is almost universally cited, it has always been our contention that ferulate should actively incorporate into the lignin polymer via oxidative coupling (radical) processes. Support for the active mechanism comes from our oxidative coupling studies on low molecular mass model compounds and from a particularly revealing study where FA-Ara (methyl 5-*O*-(*E*)-feruloyl-α-L-arabinofuranoside) was introduced into a biomimetic lignification system. That study conclusively revealed that active incorporation mechanisms produced the entire complement of ferulate/coniferyl alcohol or ferulate/coniferyl alcohol-oligomer coupling products and incorporated the ferulate intimately into the lignin. Since only ca. 10% of the ferulate could be released as ferulic acid by typical ester/ether cleaving reactions, the lower limit on the partitioning between active and passive mechanisms had to be 90% in that synthetic system. However, direct evidence supporting active incorporation in plants has been difficult to obtain and acceptance of the idea has been minimal. Traditional structural methods utilizing NMR spectroscopy were simply too insensitive to allow detection of such minor units in the lignin-polysaccharide complex (LPC). This report presents NMR proof from uniformly labeled ryegrass lignin that ferulates are indeed oxidatively coupled with lignin precursors *in vivo* and serve to cross-link polysaccharides into lignin-polysaccharide complexes.

Methods

Ryegrass was grown in an atmosphere in which the ambient levels of CO_2 were enriched in ^{13}C to a level of ca. 15%. This level is ideal for NMR since it provides the requisite sensitivity enhancement (by a factor of 15) without severe problems caused by ^{13}C-^{13}C homonuclear coupling. A dioxane/water soluble lignin fraction was isolated as previously described for maize (1992 Research Summary, p. 58). NMR experiments were performed on the USDFRC's Bruker AMX-360.

Discussion

Carbon NMR spectroscopy showed evidence for the same ferulate peaks as in the synthetic system described above (f_1 projections on the leftside of Fig. 1). Long-range C-H correlation *via* the HMBC experiment provided diagnostic correlations (Fig. 1) some of which were analogous to those evidenced in the synthetic system as indicated by overlaid symbols in Fig. 1. The most diagnostic correlation peaks, which unambiguously prove that ferulates do indeed cross-couple with lignin monomers/oligomers, are the 8-β'-coupling peaks labeled **D**. The four diagnostic correlations with the carbonyl carbon are to all of the protons in the dioxabicyclo[3.3.0]octan-2-one unit that are within 3 bonds, namely the α-, 7-, 8-, β-protons. These correlations can only arise from 8-β'-coupling reactions of ferulate radicals (8-position) with a lignin precursor radical (β-position). Correlations with peaks **B** derive from products, undifferentiated by this experiment, of 4-O-coupling; namely 4-O-β' and 5-β'/4-O-α' (phenylcoumarans) as well as the passive product, the 4-O-α' ether. The strong correlations with protons at 4.2 and 4.45 ppm clearly arise from the polysaccharide protons of the esterified primary alcohol. The shifts are in agreement with those of the C-5-acylated protons on arabinofuranosyl moieties (triangles in Fig. 1). Contributions from other attachment sites or other saccharides cannot be ruled out from this experiment.

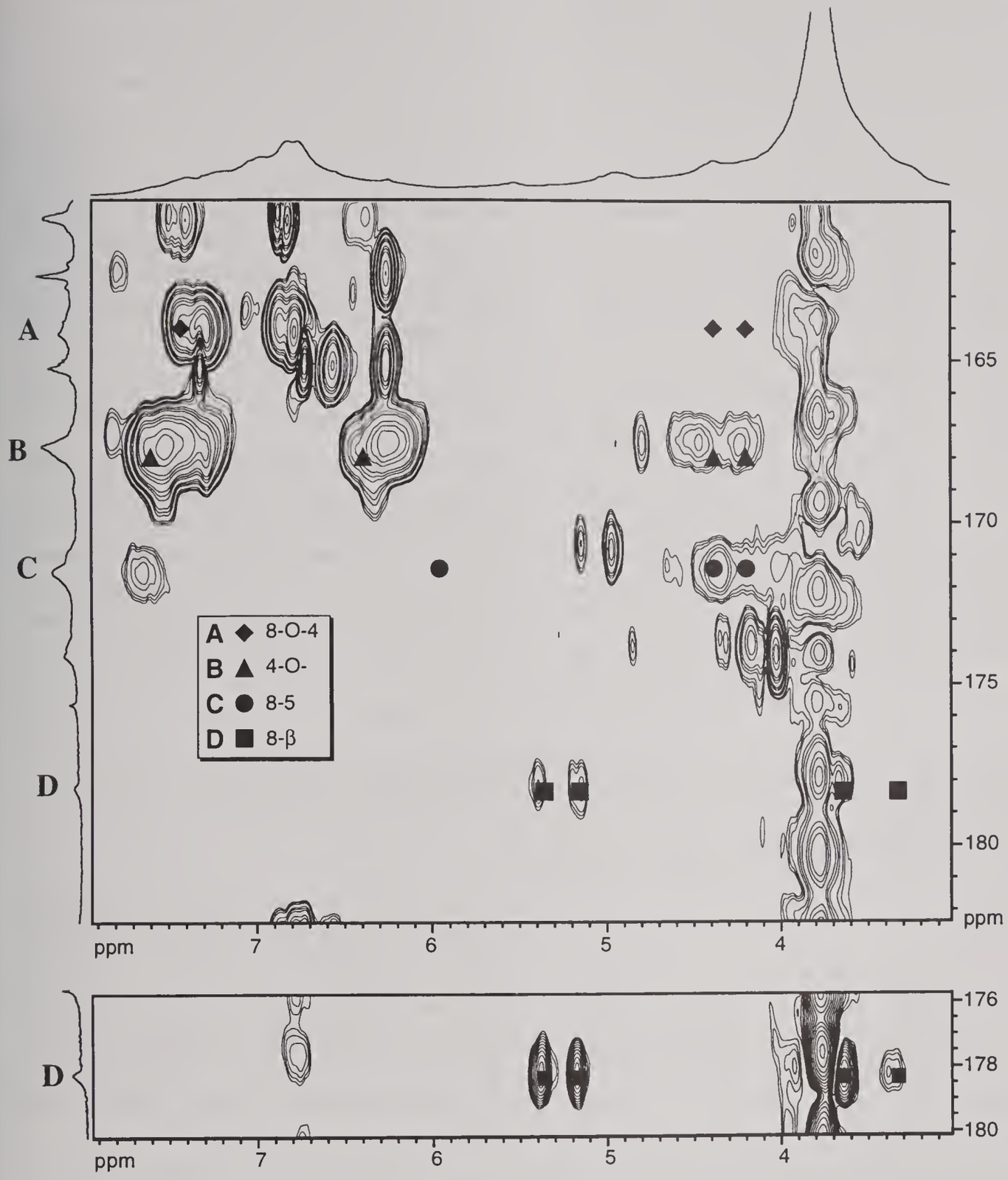


*Figure 1. Small section of a long-range C—H correlation (HMBC) spectrum with an 80 ms long-range correlation delay showing just the carbonyl carbon region. Regions are labeled corresponding to regions in a prior synthetic lignin-FA-Ara polymer (1991 Research Summaries, p. 35); **D** is the 8-β'-coupling region described in the text. Overlaid are the data for similar correlations in an FA-Ara DHP model system. The 8-β'-selection of a similar experiment run using a 100 ms long-range coupling delay which reveals the β- and 8-proton correlations more completely is shown at the bottom of this figure.*

Two important implications regarding the cross-linking process are also revealed by this correlation experiment. Firstly, while the 8-β'-coupled product is a key observation in proving that radical mechanisms are involved in ferulate incorporation into lignins, this product is the least significant in terms of cell wall cross-linking since the previously feruloylated polysaccharide moiety is released *via* internal transesterification during its production. Secondly, and more importantly, the 8-β' moiety in the LPC can arise only from coupling with a lignin unit possessing both a free-phenol and α,β-unsaturation; this can only be a coniferyl alcohol linked to another coniferyl alcohol or a lignin oligomer *via* 5-5' or 5-O-4' bonds or, most likely, free coniferyl alcohol **6** itself. The propensity of the 8-β'-coupled products in the *in vivo* system, as revealed here, is an indication that ferulate may be reacting extensively with lignin monomers and not, as in the DHP case, with lignin oligomers. This finding, although not entirely conclusive at this point, may be the first evidence that ferulate functions as a nucleation site for lignification — a site at which the first coupling reactions with lignin monomers occur and from which lignification proceeds to build up the lignin-polysaccharide complex. This theory has been advanced by us and others but has been only an interesting hypothesis until this point. Further studies aimed at understanding the differences between the synthetic and *in vivo* lignification system can shed light on temporal and mechanistic aspects of the cross-linking process and are in progress in our laboratories.

Conclusions

NMR of a uniformly labeled ryegrass lignin has unequivocally shown that ferulates do actively incorporate into lignins as we have been promoting. It is time for this mechanism to be recognized in the literature, particularly since its existence implies that all current analytical methods underestimate ferulate cross-links in plant materials.

Ferulate Cross-linking of Grass Cell Walls: Model Studies With Suspension Cultured Maize

J.H. Grabber, R. D. Hatfield, and J. Ralph

Introduction

Ferulic acid is attached as an ester to the C(O)5 hydroxyl of α-L-arabinose moieties of grass xylans. It has long been presumed that arabinoxylans are cross-linked to a limited extent by a 5-5 coupled dehydrodimer of ferulic acid. Recently our group demonstrated that oxidative coupling mechanisms form substantial amounts of 8-5, 8-8, and 8-O-4 coupled dehydrodimers in addition to the 5-5 coupled product. Therefore, previous studies have probably underestimated the importance of dehydrodimers as cross-linking molecules. Ferulates are also capable of entering the dehydrogenative polymerization process associated with lignification, thereby cross-linking arabinoxylans to lignin. Most of the ferulate is probably attached to lignin by linkages that resist acid or alkaline hydrolysis. Therefore the extent of ferulate cross-linking in lignified tissues has probably been underestimated by solvolytic methods. Our objective was to determine the potential extent and type of cross-linked structures formed by ferulates in primary cell walls of grasses. This summary describes how ferulate cross-linking was affected by manipulating ferulate deposition, peroxidase activity, and cell wall lignification.

Methods

Nonlignified suspension cultures of maize (*Zea mays* L.) were grown under normal conditions or with 2-aminoindan-2-phosphonic acid (AIP, an inhibitor of phenylalanine ammonia lyase) to reduce the feruloylation of arabinoxylans. A dilute solution of hydrogen peroxide was added to suspensions of nonlignified walls to determine how extensively ferulate monomers can be coupled into

dehydrodimers by wall-bound peroxidase. The potential extent and type of ferulate cross-links in lignified walls was investigated with dehydrogenation polymer-cell wall (DHP-CW) complexes. The complexes were formed by using wall-bound peroxidase and *in situ* generated hydrogen peroxide to polymerize a mixture of coniferyl and sinapyl alcohol into a suspension of nonlignified walls. Cell walls were subjected to saponification and high temperature alkaline hydrolysis to sequentially release ester- and ether-linked hydroxycinnamic acids. These acids were quantified by GC-FID.

Results and Discussion

Growing cultures with 0 to 50 µM AIP reduced total ferulate concentrations in cell walls from 17.2 mg g^{-1} to 4.3 mg g^{-1} and increased the proportion of dehydrodimers to total ferulate from 15 to 24%, indicating that maize cells compensated for reduced ferulate deposition by increasing dehydrodimer formation. In a separate study, dimerization of ferulate monomers by wall-bound peroxidase was increased 260% by adding dilute hydrogen peroxide to nonlignified cell walls (Table 1), suggesting that dehydrodimer formation was limited by the availability of hydrogen peroxide. About 45% of the dehydrodimers formed by wall-bound peroxidase were coupled by 8-5 linkages, with 8-8, 8-O-4 and 5-5 coupled dehydrodimers each comprising 15 to 20% of the total. The quantity of ferulates that could be released by saponification was reduced by 90% when cell walls were synthetically lignified to form DHP-CW complexes (Table 2). Only 40% of the ferulate incorporated into lignin was recovered following hydrolysis of ether linkages, suggesting that most of the ferulate was coupled to lignin by stable carbon-carbon linkages formed by oxidative coupling mechanisms.

Conclusion

These results indicate that: 1) primary cell walls become extensively cross-linked by ferulic and dehydrodiferulic acids during lignification, and 2) only some of the ferulates in lignified tissues are measurable by current solvolytic methods.

Table 1. Effect of hydrogen peroxide treatment on the formation of dehydrodiferulates by wall-bound peroxidase. Ferulate deposition into cell walls was reduced by growing cultures with AIP, a specific inhibitor of phenylalanine ammonia lyase.

		Ferulate	Ferulate Dehydrodimers				Total
AIP	H_2O_2	monomers	8-8	8-5	8-O-4	5-5	ferulates
- - - µM - - -		- - - - - - - - - - - - - - - - - - mg g^{-1} cell wall - - - - - - - - - - - - - - - -					
0	0	14.60	0.57	1.22	0.34	0.45	17.20
0	200	8.83	0.88	2.78	1.25	1.13	14.87
40	0	3.84	0.33	0.66	0.17	0.27	5.27
40	200	2.26	0.33	1.09	0.33	0.35	4.37

Table 2. Incorporation of ferulate monomers (FM) and dehydrodimers into lignin. Wall-bound peroxidase and *in vitro* generated hydrogen peroxide were used to polymerize hydroxycinnamyl alcohols (HCA) into cell walls isolated from nonlignified suspension cultures of maize. Cultures were grown with AIP to reduce the deposition of ferulate esters into cell walls.

Treatment			Ferulates released by saponification of ester linkages					Ferulates released by hydrolysis of ether linkages				
AIP	HCA	Klason Lignin	FM	8-8	8-5	8-O-4	5-5	FM	8-8	8-5	8-O-4	5-5
µM	- mg g^{-1} of cell wall -											
0	0	10	10.44	1.24	3.61	1.55	1.20	0.14	0.26	0.04	0.01	0.04
0	94	113	1.14	0.37	1.11	0.31	0.10	2.93	1.10	1.29	0.99	0.50
10	0	8	3.79	0.61	2.21	0.81	0.65	0.06	0.14	0.01	0.01	0.02
10	97	105	0.24	0.10	0.06	0	0	1.25	0.52	0.65	0.43	0.30

Ferulate Cross-Linking Limits Degradation of Lignified Grass Walls by Fungal Hydrolases

J.H. Grabber, R.D. Hatfield, and J. Ralph

Introduction

During lignification of grass fiber, ferulate esters on arabinoxylans become coupled to lignin. Ferulate cross-linking of arabinoxylans to lignin may limit the enzymatic degradation of grass fiber, reducing the bioconversion of structural polysaccharides into metabolizable energy for livestock or into ethanol for fuel. This summary describes the use of dehydrogenation polymer-cell wall (DHP-CW) complexes to model how ferulate cross-links affect the degradation of lignified grass fiber by fungal hydrolases. Previous work (see 1993 Research Summaries) has demonstrated that DHP-CW complexes accurately model interactions among matrix components in lignified grass walls .

Methods

Wall-bound peroxidase and *in situ* generated H_2O_2 were used to form dehydrogenation polymers of coniferyl alcohol within nonlignified walls isolated from suspension cultures of maize (*Zea mays* L.). Cell walls with 17 and 4.7 mg g^{-1} of ferulates were synthetically lignified to Klason lignin concentrations ranging from 50 to 160 mg g^{-1}. Ferulate concentrations in cell walls were reduced by growing cultures with 2-aminoindan-2-phosphonic acid (AIP, an inhibitor of phenylalanine ammonia lyase) or by selectively methylating wall ferulates with diazomethane prior to complex formation. Samples of DHP-CW complexes (100 mg) were suspended in 10 mL of acetate buffer (20 mM, pH 4.8) and incubated at 39°C with hydrolases from *Trichoderma reesei* (4 µL of Celluclast, NOVO) and *Aspergillus niger* (4 ML of Viscozyme L, NOVO). During the course of enzyme hydrolysis, samples were centrifuged and an aliquot of the supernatant was analyzed colorimetrically for total sugars and uronic acids. Supernatant samples were hydrolyzed with 2 N trifluroacetic acid and neutral sugars were quantified by Dionex HPLC. Residues remaining after enzyme hydrolysis were collected on tared glassfiber filters (0.9 µm retention).

Results and Discussion

A 70% reduction in ferulate cross-links increased the degradability of DHP-CW complexes by 20% (Table 1 and Figure 1 and 2). Improved degradability was due only to reduced ferulate cross-linking because similar results were observed with two diverse methods for reducing ferulate cross-links. Ferulate cross-links reduced the release of all neutral and acidic sugars from DHP-CW complexes, particularly that of xylose and arabinose (Figure 3).

Conclusion

Our results indicate that selection or genetic engineering of grasses for low feruloylation of arabinoxylans will significantly improve the degradability of lignified walls by fungal hydrolases. In 1995 we will determine if reducing ferulate-cross-linking of DHP-CW complexes will improve the degradation of lignified fiber by rumen microorganisms.

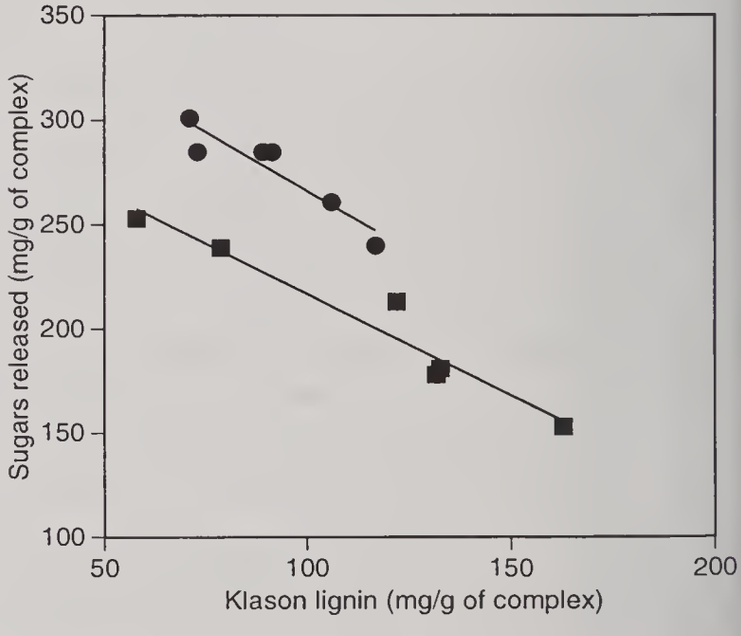


Figure 1. Release of sugars from DHP-CW complexes after a 6 h incubation with fungal hydrolases. Complexes contained 15.5 mg/g (■) or 4.6 mg/g (●) of ferulate cross-links between arabinoxylans and lignin. Ferulate cross-links were reduced by methylating the phenolic hydroxyl of wall ferulates prior to complex formation.

Table 1. Fungal hydrolase degradation of DHP-CW complexes with normal or low levels of ferulate cross-links. Ferulate cross-links were reduced by growing maize cultures with AIP (to reduce the deposition of ferulate esters into cell walls) prior to complex formation.

Ferulate cross-links	Klason lignin	Neutral sugars released 6 h	72 h	Degradation at 72 h
	- - - - - - - - - - - - - - - - mg g^{-1} of complex - - - - - - - - - - - -			
15.7	155	171	428	597
4.5	151	250	507	717

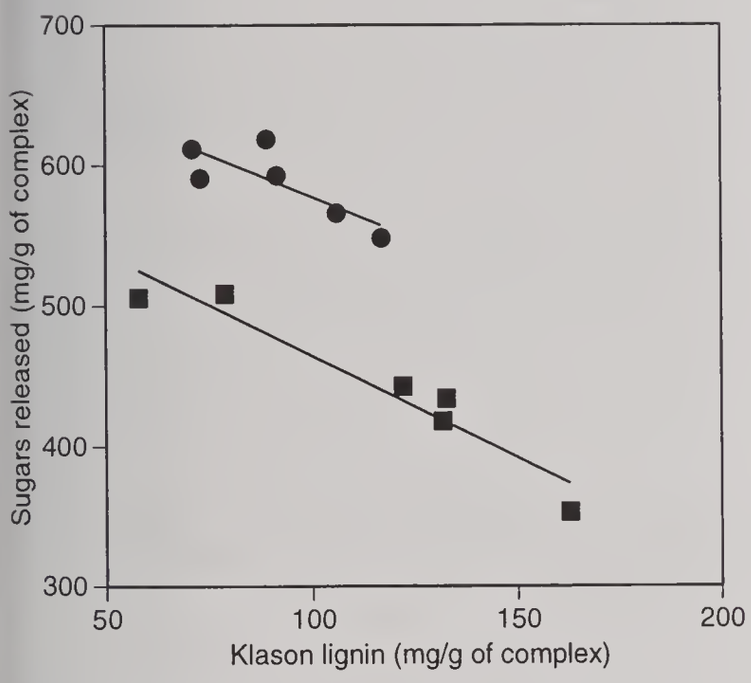


Figure 2. Release of sugars from DHP-CW complexes after a 72 h incubation with fungal hydrolases. Complexes contained 15.5 mg/g (■) or 4.6 mg/g (●) of ferulate cross-links between arabinoxylans and lignin. Ferulate cross-links were reduced by methylating the phenolic hydroxyl of wall ferulates prior to complex formation.

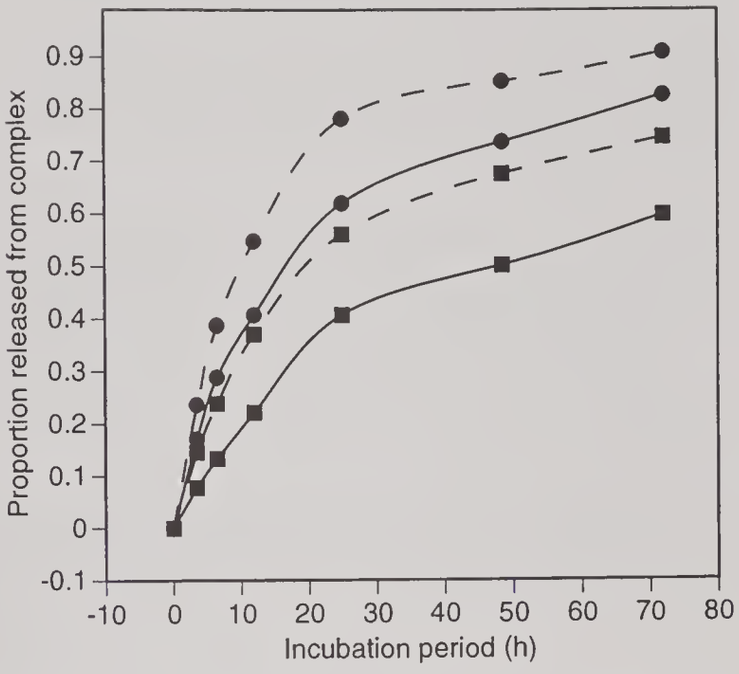


Figure 3. Release of xylose (—) and arabinose (- -) during incubation of DHP-CW complexes with fungal hydrolases. Complexes contained normal (■) or low (●) levels of ferulate cross-links.

Reactions of Lignin Model Compounds With Acetyl Bromide

F. Lu, and J. Ralph

Introduction

Selective methods for cleavage of benzyl ether linkages in lignin have recently become a focus of our group in attempts to distinguish active (free radical) and passive (nucleophilic attack on quinone methide intermediates) incorporation of ferulate into lignin during plant cell wall development. We recently showed that bromotrimethylsilane is an excellent selective reagent for benzyl aryl ether cleavage but is difficult to apply to real-world plant materials. Recently we have become interested in the possibility of AcBr to more easily effect similar reactions in a broader range of solvents. This paper reports an investigation into the reactions of lignin model compounds with AcBr in good lignin solvents

aimed at better understanding the reaction and developing new methods for analysis of benzyl ether linkages in lignin. An unexpected spin-off is the synthetic utility of these reactions.

Methods

Lignin model compounds **1a**, **1b**, **4b**, **6a** and **6b**, shown in Figure 1, were synthesized according to the standard methods. Compounds **2a-5b** and **7b-11** were obtained as described below by the treatment of lignin model compounds with AcBr and characterized by their ^{1}H and ^{13}C NMR spectra. NMR data are deposited in the recently released USDFRC/USFPL NMR database (1993 Research Summaries, p. 56).

AcBr treatment of lignin model compounds:— To 0.05 to 0.20 mmoles of lignin model compound in 2 ml of solvent (acetic acid, 1,4-dioxane, or DMF) was added 2.0 to 6.0 equivalents of AcBr. The mixture was kept at room temperature with gentle stirring and monitored by t.l.c. Reaction was terminated by addition of water (for DMF) or evaporation of solvents (for 1,4-dioxane and acetic acid). When the solvent was DMF, water was added and the product was extracted with ethyl acetate, washed with water, saturated NH_4Cl (2x). The organic phase was dried over anhydrous $MgSO_4$ and evaporated under reduced pressure. The weighed product was characterized directly by NMR. When acetic acid or 1,4-dioxane was used as solvent, the solvent was removed on a rotary evaporator and evaporated several times following addition of acetone to remove the solvent completely for NMR characterization.

Discussion

From the results summarized in Table 1, it is evident that the primary γ-OHs of the model compounds were readily acetylated by AcBr treatment. Although the secondary benzylic hydroxyls, when treated with AcBr in AcOH or 1,4-dioxane, were also acylated, they subsequently formed α-bromo derivatives in almost quantitative yield. Phenolic OHs were acylated with AcBr in 1,4-dioxane more slowly than primary OHs. When the AcBr treatments of model compounds **1a, 1b** were performed in DMF, the major products were peracetate derivatives, although α-bromides were also obtained.

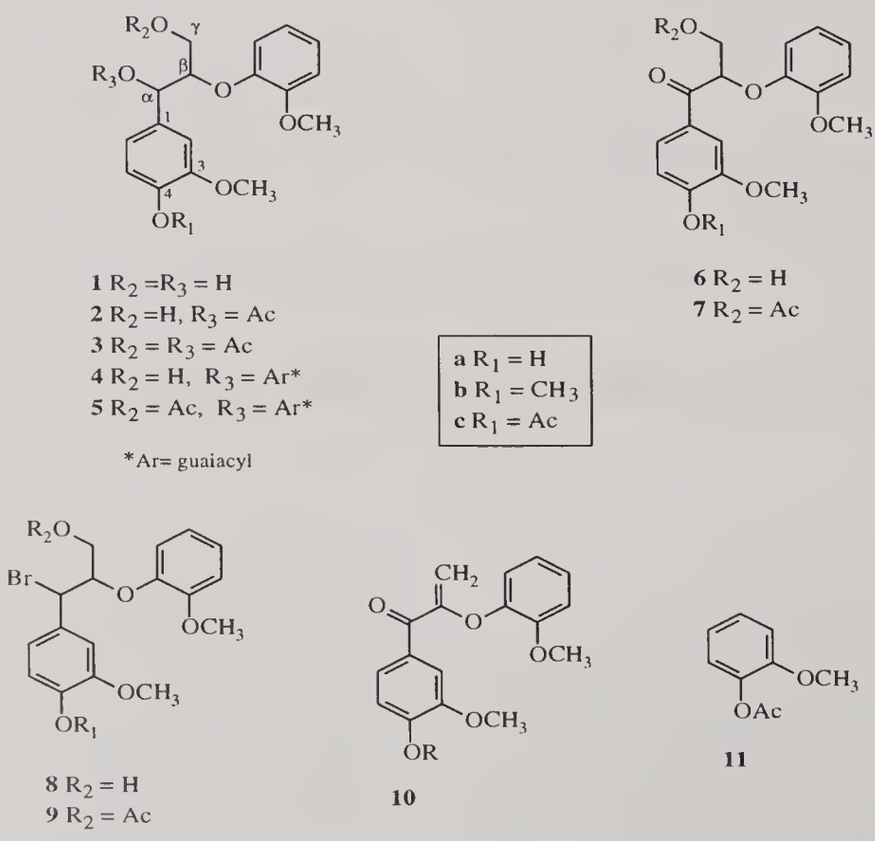


Figure 1. Lignin model compounds and products resulting from AcBr treatment.

The benzylic hydroxyls of model compounds **1a** and **1b** were completely converted to acetylated α-bromo derivatives **9b** and **9c** after AcBr treatment in acetic acid or 1,4-dioxane (Table 1). To our knowledge, this finding has not been reported previously and makes AcBr synthetically useful for selective bromination of benzylic hydroxyls in the presence of other primary or secondary hydroxyls. Additionally, if stereochemical integrity is not a problem, AcBr may be used to selectively acetylate primary or phenolic hydroxyls in the presence of benzylic hydroxyls because the resulting benzylic bromide can be readily hydrolysized (acetone/water) back to the benzylic hydroxyls without impacting the acetates.

Benzyl aryl ethers (e.g. model compound **4b**) were selectively cleaved quickly and cleanly, without impacting the β-ether after > 12 h. The NMR spectra of the products showed that the α-bromo acetylated product **7c** and 2-methoxy phenyl acetate **9a** were formed quantitatively from the AcBr treatment of compound **4b** in AcOH or 1,4-dioxane. This exciting finding means that, with the application of appropriate protection strategies, AcBr could be used for analysis of benzyl aryl ether linkages present in lignin. AcBr should also be useful for releasing those hydroxycinnamic acids (ferulic acid and *p*-coumaric acid) which are bound to the α-position on the sidechain of lignin structural units. Such applications are being evaluated in our laboratory.

Conclusions

Acetyl bromide provides a more flexible approach to cleaving benzyl aryl ethers and should allow us to develop methods for determining the ferulates that are passively incorporated into lignins. This study also improves the understanding of a lignin analytical method which utilizes AcBr and demonstrates the synthetic utility of the reagent.

Table 1. The products from the reactions of lignin model compounds with acetyl bromide in some lignin solvents.

Model Compound	Solvent	Time	AcBr (eq.)	Products (yield % or ratio)
1a	Acetic acid	60 min	6.0	**9c** (>98)
		O/N	6.0	**9c** (>98)
	1,4-dioxane	60 min	6.0	**9c** (8) +**9a** (92)
		O/N	6.0	**9c** (>98)
	DMF	60 min	6.0	**9c** (17)+ **3c** (58)+ **3a**(17)+**2a**(6)
		O/N	6.0	**9c** (18) + **3c** (76) + **3a** (6)
1b	Acetic acid	90 min	4.0	**9b** (59) + **2b** (41) + **3b** (trace)
		O/N	4.0	**9b** (>98) + **3b** (trace)
	1,4-dioxane	90 min	4.0	**9b** (>98) + **3b** (<1)
		O/N	4.0	**9b** (>98) + **3b** (trace)
	DMF	90 min	4.0	**3b** (80) + **2b** (20)
		O/N	4.0	**3b** (75) + **9b** (25)
4b	Acetic acid	8 min	4.0	**9b** (>98) + **11** (>98)
	1,4-dioxane	8 min	4.0	**9b** (>98) + **11** (>98)
	DMF	30 min	4.0	**5b** (>97)
6a	Acetic acid	10 min	2.0	**7b**(>95) + **10b** (< 5)
	1,4-dioxane	10 min	2.0	**7b**(>95) + **10b** (< 5)
	DMF	10 min	2.0	**7b** (100)
6b	Acetic acid	10 min	4.0	**7a** (>94)+ **10a** (<6)
	1,4-dioxane	10 min	4.0	**7a** (93)+ **10a** (3.5) + **6b** (3.5)
	DMF	10 min	4.0	**7a** (98) + **7c** (2)

Thioacidolysis Products of Incomplete β-Ether Cleavage

J. Ralph, J.H. Grabber, and B. Haggerty

Introduction

Thioacidolysis has become a widely used tool in lignin characterization. It is based on the ability of the reagents to cleave ether linkages in lignin; ether linkages are the major linkages by which lignin moieties are hooked to each other and are the easiest to cleave chemically. Thioacidolysis has become one of the more powerful diagnostic tools, particularly with the Raney nickel post-treatment for characterizing dimer fragments,

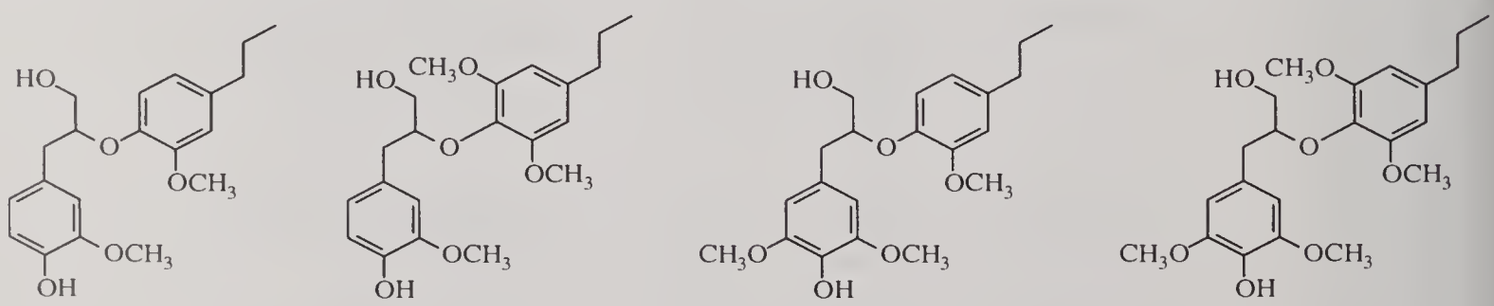
During our applications of this technique to analyzing forage samples, biomimetically lignified suspension cultures, and cell wall preparations, we noted the appearance of peaks that were not previously identified. Their mass spectra (from GC-MS) suggested that they were β-ether dimers. Since the appearance of these dimers among thioacidolysis products would imply incomplete cleavage of β-ether units in lignin (and therefore result in low values for such units in quantitation), we sought to firmly identify these compounds by independently synthesizing authentic compounds and comparing their GC retention times and mass spectra with those compounds from the plant materials.

Methods

The four compounds (Figure 1) representing all the possible β-ether products which would result from incomplete thioacidolysis of a syringyl/guaiacyl lignin were synthesized by modifying normal lignin model β-ether strategies. The α-CH_2 groups were generated from α–hydroxy parents via hydride reduction of their quinone methides. The compounds were individually analyzed by GC-MS. Proof of their identity with cell wall thioacidolysis products was obtained by co-injection of plant thioacidolysis products with the synthesized compounds.

Discussion

There are four possible dimeric compounds that can result from incomplete β-ether cleavage after thioacidolysis/Raney nickel desulfurization of a mixed guaiacyl/syringyl lignin (Fig. 1). The entire set of these compounds can be detected by GC and GC-MS (Fig. 2) of a range of plant materials using conditions that appear to be identical to those in the literature. We have not carried out our own optimization studies, and it is possible that our conditions are not identical to those of the developers of the method. It is interesting, Figure 2, that all four homologues are seen in similar concentrations. This has two implications. Firstly, it demonstrates that all of the possible β-ethers cleave uniformly well (or uniformly poorly) and that syringyl-guaiacyl differences are unimportant. Secondly, the very detection of these units implies that there are significant amounts of β-ether trimer (or β-ether dimer terminal units) in the lignins studied — thioacidolysis will cleave only ether bonds, so



G-G
$C_{20}H_{26}O_5$
(346)

G-S
$C_{21}H_{28}O_6$
(376)

S-G
$C_{21}H_{28}O_6$
(376)

S-S
$C_{22}H_{30}O_7$
(406)

Figure 1. The four possible dimeric compounds that can result from incomplete β-ether cleavage after thioacidolysis/Raney nickel desulfurization of a mixed guaiacyl/syringyl lignin. Each of these compounds was independently synthesized for authentication. S = syringyl, G = guaiacyl. The nominal molecular weights are in parentheses.

these dimers could only be released for detection if they in turn were attached by β-ethers at each end. The existence of such trimeric units is not surprising since the β-ether linkage is the major one in lignins and others have reported significant levels of β-ether trimeric units, but the amounts we appear to get here, e.g. Figure 2, may indicate that most of these trimeric units leave the middle β-ether bond uncleaved. Is it then easier to cleave β-ether bonds when the neighboring β-ether is attached to the rest of the lignin molecule by non-β-ether units? Fascinating basic questions regarding the mechanism and relative cleavage rates of the various β-ethers in lignin will not be pursued by our group — we must leave it to other interested parties to explore what further information can be learned about lignin structures by analysis of these β-ether dimers. The aim here is simply to caution researchers using thioacidolysis to be on the lookout for these dimeric β-ether products and, if necessary, adjust their quantitative figures accordingly.

Conclusions and Recommendations

Although their amounts varied considerably among samples, all of the four β-ether dimers were located in several plant thioacidolysis extracts and, in some cases (e.g. Fig. 2) were quite abundant. This implies that β-ethers were not fully cleaved under our conditions. While we do not claim that our procedure is optimal or that these products will arise in every thioacidolysis run, we advise researchers to be aware of these products so that their quantitative data can be adjusted — β-ether units are typically identified in the monomer fraction but accounting for these peaks that may appear in the dimer fraction is strongly advised.

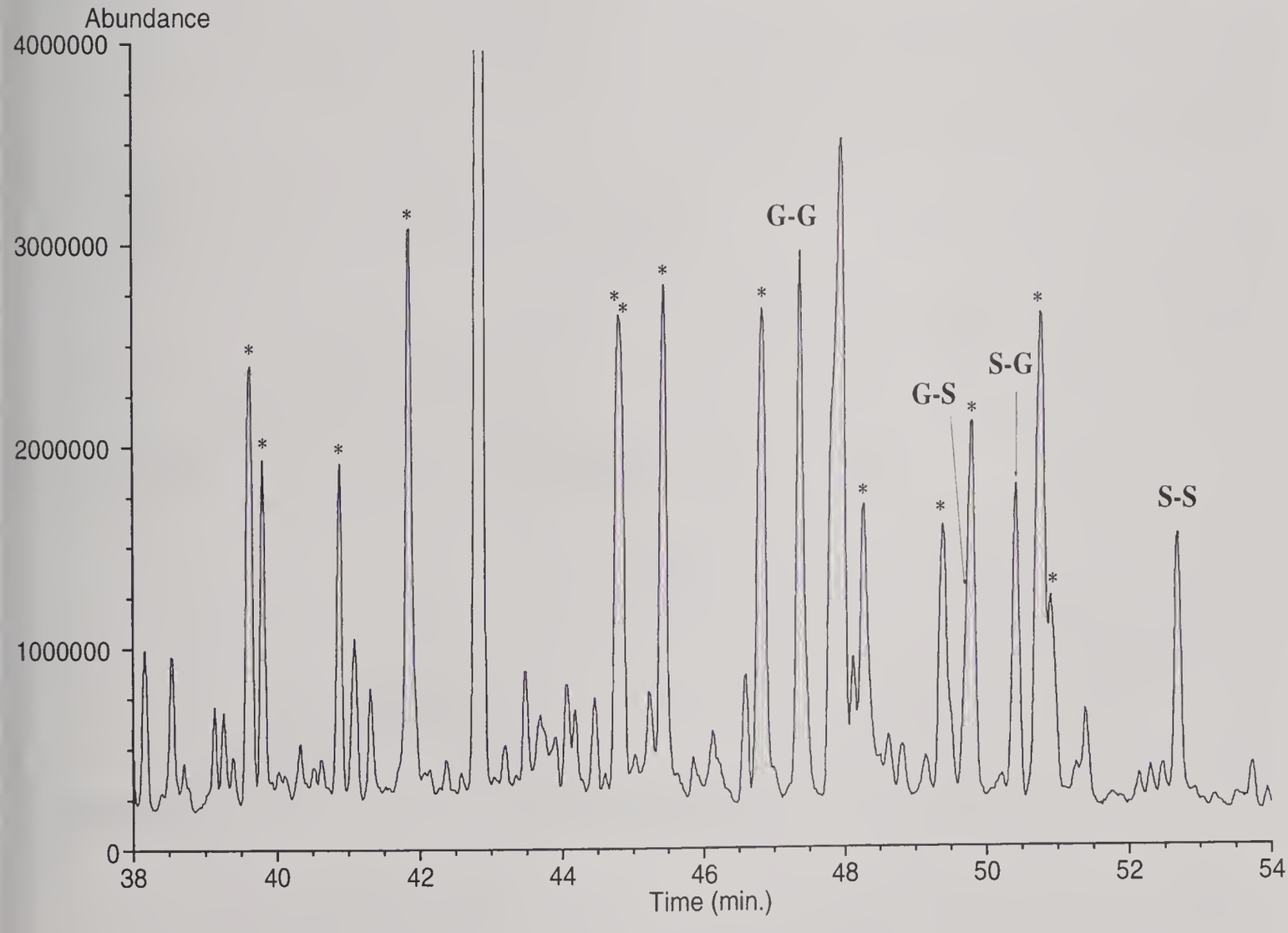


Figure 2. GC-MS of a willow thioacidolysis product showing the four β-ether dimers as well as the other more commonly identified dimers (asterisks).

SDS Solubilized Lignin Complexes From Alfalfa Stems

R.D. Hatfield, K. Andrewartha, and B.A. Stone

Lignin is a macromolecule polymerized in wall matrices of fully expanded cells to provide structural strength. As lignin formation continues, other wall components can become crosslinked to it either through passive (addition to quinone methides) or active (free radical addition) mechanisms to form large complexes. In order to understand the formation of these interactions, it is necessary to isolate lignin complexes in a form amenable to further analysis. This work describes general characteristics of lignin complexes solubilized by sodium dodecyl sulfate extraction of alfalfa stem walls.

Materials and Methods

Alfalfa (*Medicago sativa*, Siriver) stems were separated from the leaves and divided into three regions before freeze drying: lower (LN) 15-20 cm, middle (MN) 15-20 cm, and top (TP)10-15 cm. Dried stems were ground to pass a 0.5 mm screen before extraction with cold buffer, hot 80% ethanol, acetone, and chloroform:methanol (2:1). Extracted samples were dried over P_2O_5 for 3-4 days, then ball milled for 48h. Recovered milled walls were first extracted with EDTA overnight followed by two extractions with hot SDS. The first SDS extraction was with continuous stirring for 2h at 90°C, centrifuged (5000 Xg) and the supernatant decanted. For the second extraction fresh SDS was added and heated to 90°C, the heat turned off and the sample continuously stirred overnight. Samples were centrifuged, the supernatant decanted and combined with the first extract. SDS was partially removed by precipitation with KCl. Solubilized complexes were further fractionated by dioxane extraction and size exclusion chromatography. Isolated fractions were analyzed for total carbohydrate, proteins, and lignin.

Results and Discussion

Treatment with hot SDS solubilized 10-15% of ball milled cell walls (Table 1). These extracts contained between 40-60% lignin depending upon the maturity of the stem material. A portion (500 mg) of the SDS extracts was further divided into dioxane soluble and insoluble fractions. Dioxane (96:4, dioxane:H_2O) solubilized between 27 and 38% of the original extract. For both LN and MN samples, dioxane extracts were 90% lignin, but TP samples were only 70% lignin with the remaining material mainly carbohydrate.

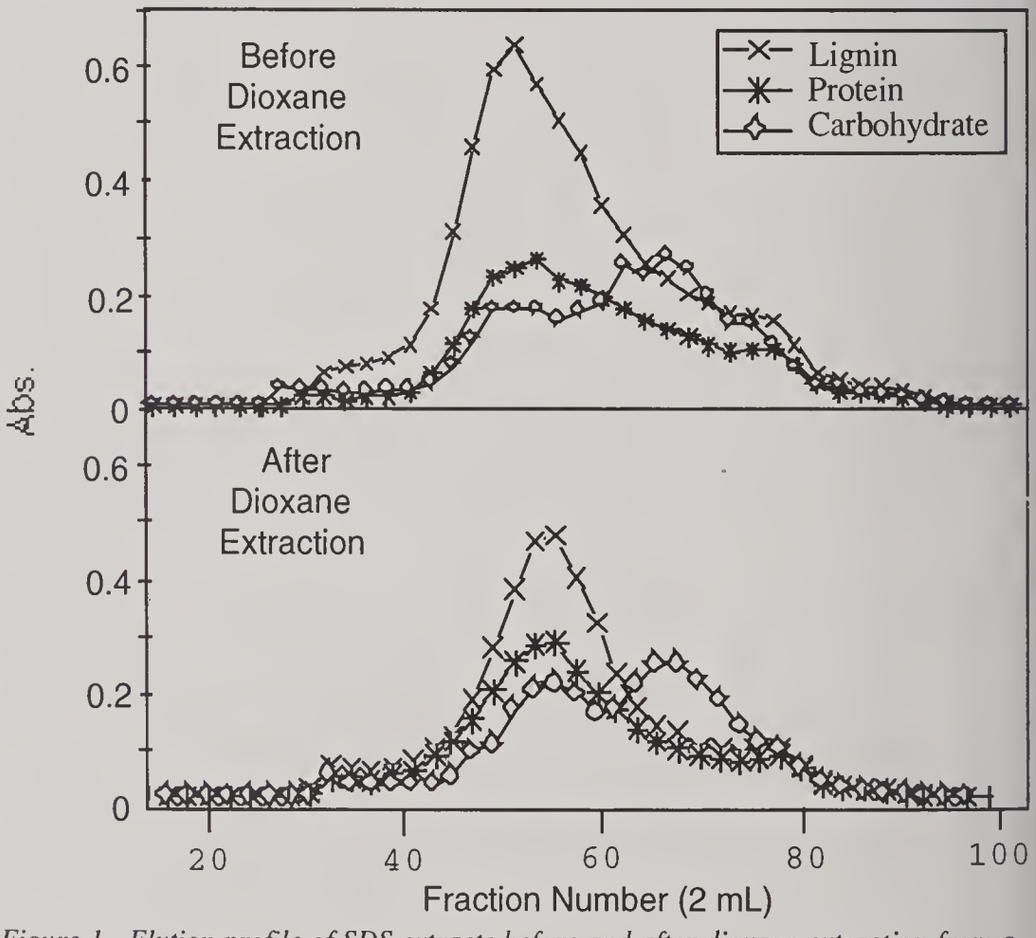


Figure 1. Elution profile of SDS extracts before and after dioxane extraction from a Sephacryl-400 column (1.5 X 120 cm). Ammonium acetate (200 mM) was used as elution buffer at a flow rate of 12 mL h^{-1}.

Subsamples of the SDS extracts, before and after dioxane treatment, were fractionated on a Sephacryl-400 column (Fig 1). For all samples, three major fractions were collected: Frac 2 (high 75-200kD molecular mass, Fractions 45-62), Frac 3 (medium, 40-75kD molecular mass, Fractions 63-75), and Frac 4 (low, 10-40 kD molecular mass, Fractions 76-85). The distribution of materials among the three fractions did not show any appreciable shift among fractions after dioxane treatment; however it was apparent that a lignin-rich fraction was removed from Frac 2. All other fractions remained unaltered. Neutral sugar composition of the fractions was similar, each containing xylose, arabinose and glucose as the major constituents. Frac 2 was unique in that it contained the largest proportion of protein of the three column fractions, whereas Frac 3 contained the highest proportion of carbohydrate. Amino acid analysis of the proteins in all fractions indicated that there was no unique composition present. The amino acid composition and molar proportions were similar to initial wall preparations. It would appear that the proteins involved in these complexes were not specifically structural in nature but resembled other wall bound proteins, such as peroxidase or hydrolases.

Treatment of Frac 2 with trypsin/chymotrypsin for several hours resulted in only a small amount of degradation of the protein in this fraction. This partial degradation did not result in a major shift in the elution profile indicating that the main portion of the complex remained intact. It would appear, however, that even though these complexes were quite soluble, degradation was limited most likely due to crosslinking between the protein and lignin. Further analysis would be required to determine the chemical nature of these interactions. This may be a difficult task due to the large number of protein types bound in the complex with different modes of attachment – some that are active and others that are passive in nature.

Treatment of Frac 3 (rich in xylans) with a purified xylanase produced major shifts in its elution profile. The bulk of the carbohydrate shifted to the low molecular mass fraction region (Frac 4), while the remaining lignin-protein complex containing some carbohydrate shifted in the opposite direction to higher molecular mass (Frac 2) as well as some becoming insoluble. This would indicate that the xylans within this fraction aid in maintaining solubility of the whole complex. Whether complexes of this type would be degraded by rumen microbes remains to be determined. It would seem that the high solubility of these complexes might make them prone to washing out of the rumen before complete degradation.

Table 1. Summary of SDS extraction of alfalfa stems.

Stem Region	% of cell wall	Lignin g g^{-1} of extract
LN	10.4	0.605
MN	15.4	0.586
TP	13.8	0.424

Determining the Pectic Polysaccharide Content of Legumes

R.D. Hatfield, and R.R. Smith

Introduction

Pectic polysaccharides are rapidly degraded and utilized by rumen microbes. In previous work, it had been shown that alfalfa stems could contain up to 30% of their cell walls as pectic materials. It would be of nutritional advantage if alfalfa or other forage legumes contained significant amounts of pectic materials. Work was undertaken to determine the genetic variation for pectic materials in a diverse population of alfalfa entries. The population was kept relatively small in order to investigate the influence of harvest date, plant maturity, plant part (leaves vs. stems), and drying method on total pectic materials.

Materials and methods

Legume plants utilized for this study included 11 *Medicago sativa*, 3 *Medicago falcata*, 1 red clover, and 1 trefoil. All plants were harvested from replicated field plots at Arlington, WI. Two stages of development, bud and 50% bloom, were collected for each of the first and second harvest. The actual dates of harvest varied in order to obtain the proper stage of development. Plots that were harvested as bud or 50% bloom in the first cutting were also harvested at the same stage of development in the second harvest. Therefore, the second harvest extended well into mid summer for some plots. Plants were either field, hot air (55°C), or freeze dried before being separated into stems and leaves. All samples were ground (Udy mill) to pass a 1mm screen. Approximately 150 mg of each sample was weighed into a 15 mL screw-capped centrifuge tube, extracted four times with 80% ethanol, once with chloroform:methanol (2:1), and once with acetone before air drying. At the same time, samples were weighed into crucibles for dry matter and ash determinations. Extracted samples were solubilized and hydrolyzed with the two step, sulfuric acid method (1st 12M room temp., 2nd 1.6M at 100°C). Subsamples were removed for total uronosyl and for neutral sugar determinations.

Four samples, a high leaf and high stem and low leaf and low stem, along with 2 standard alfalfa leaves, were subjected to traditional methods of pectic polysaccharide extraction. This allowed us to evaluate the overall efficiency of evaluating total pectic content by the methods described above.

Results and Discussion

Total pectic material for each plant sample was estimated from the sum of the total uronosyl assay and the combined amounts of rhamnose, galactose, and arabinose. These neutral sugars are associated almost entirely with pectic polysacchrides in legume plants. Table 1 lists means across field replicates, drying treatment and maturities for all entries. It can be seen that total pectin on a dry matter basis varies among the different entries. Although there are small differences, generally the trends seen in harvest 1 are the same as in harvest 2. Total pectic estimates for stems in harvest 2 are slightly higher than the leaves. It is important to point out that part of the total uronosyls measured by the methods used in these investigations contain contributions from the non-pectic uronosyls (glucuronosyl) found attached to xylans.

Table 2 summarizes the comparison between estimates of pectic polysaccharides based on the total CW hydrolysis procedure and a traditional extraction procedure. Generally the low pectic samples were close in the two methods, whereas the high samples were not as close. This was particularly true for the stem sample. As mentioned above, glucuronosyls will contribute to this error, but for the high stem it is also likely that the extraction procedures used did not fully remove all of the pectic polysaccharides from the wall matrix resulting in an underestimation. These results would suggest that sufficient genetic variation exists among alfalfa germplasm to merit further research relative to selection of plants with higher pectic polysaccharide concentration.

Table 1. Means of total pectic material estimates across all treatments. All values are on a percent dry matter basis.

Entry	Harvest I				Harvest II			
	Leaf	R	Stem	R	Leaf	R	Stem	R
Blazer	13.13	1	12.52	2	11.88	1	13.40	10
M. sativa C	13.72	2	13.62	10	12.39	7	12.59	1
M. sativa A	13.79	3	13.93	12	12.35	5	13.50	11
Cornel Hi Pro	13.79	4	13.34	8	11.98	3	13.14	6
M. sativa B	13.80	5	13.87	11	13.05	11	12.98	3
Hill Lo Lig	14.05	6	12.05	1	12.48	8	13.01	4
Hill Hi Lig	14.23	7	12.48	3	12.67	9	13.27	8
Saranac	14.38	8	13.37	9	12.32	4	13.23	7
Can Lo Sol Pro	14.54	9	13.22	6	13.23	13	14.43	14
M. falcata A	14.78	12	14.43	13	13.59	16	14.68	15
Can Hi Sol Pro	14.86	13	13.30	7	11.88	2	12.90	2
Vernal	15.02	14	12.75	4	12.89	10	13.27	9
M. falcata C	15.18	15	14.48	14	13.30	14	14.20	13
M. falcata B	15.56	16	14.67	16	13.39	15	14.01	12
Red clover Mar	14.60	11	14.48	15	13.11	12	16.29	16
Trefoil Witt	14.55	10	13.12	5	12.36	6	13.02	5

R= Rank within each column.

Table 2. Summary of pectic polysaccharides estimated from the extraction procedure (Ext.) compared to the total hydrolysis procedure (Hydrol.). All values are percent dry matter basis.

Sample	Total Uronosyls (Ext.)[b]	Total Uronosyls (Hydrol.)[b]	Total NSa (Ext.)	Total NS (Hydrol.)	Total Pectic Materials (Ext.)	Total Pectic Materials (Hydrol.)
Low Leaf	5.54	5.10	5.53	4.90	11.07	10.00
High Leaf	7.15	11.40	6.78	6.80	13.93	18.20
Low Stem	3.59	4.50	3.97	3.10	7.56	7.60
High Stem	4.85	11.00	4.98	7.40	9.83	18.40
Std Leaf A	5.57	7.50	5.76	4.70	11.33	12.20
Std Leaf B	5.94	7.50	5.51	4.70	11.45	12.20

[a]NS = neutral sugars

[b]Indicates the type of procedure used for particular components or total pectic material.

Modifications of Fiber Methods Affecting Forage Analysis

D.R. Mertens

Introduction

The best way to determine the variability of a method is to conduct a collaborative study in which the method is used by several laboratories that are following the same set of instructions. The Association of Official Analytical Chemists (AOAC) often sponsors these tests and uses the results of collaborative studies to determine if a method can be approved for routine and official use. However, labs often modify methods intentionally or unintentionally. Although modifications can cause errors in all methods, they are especially harmful when the component being measured is defined by the method. For example, fiber does not represent a distinct or homogeneous chemical entity. Each type of fiber is defined by the method used to measure it (crude fiber, acid detergent fiber, neutral detergent fiber, etc.). Modifications of fiber methods have the potential of creating a new type of fiber that may or may not compare to the original. Thus, procedure modifications may be a major source of variation in the reproducibility of fiber analyses among laboratories.

Materials and Methods

Of the approximately 150 labs participating in the National Forage Testing Association (NFTA) certification program for 1993, approximately 110 provided information about acid detergent fiber (ADF) methods and 80 provided information about neutral detergent fiber (NDF) methods. Data sets actually available for assessing the effects of individual responses or combinations of responses on fiber results were often less than these maximum numbers of observations because answers were not always provided for every question.

The General Linear Models procedure of SAS was used to statistically analyze the effects of procedure modifications on fiber results. Initially the full model compared the fiber value for a method to all question responses for that method. Non-significant responses were removed from the statistical analyses until a final set of responses remained that had a significant impact on results. Follow-up responses will be obtained from some labs to create a more complete data set and permit a more thorough analysis of factors causing the differences in fiber results among laboratories.

Modifications of the NDF method also were compared using chemical analyses from the first quarter, 1993. Three modifications that were used by at least eleven laboratories were compared. The results of three labs were removed as outliers because they deviated excessively from other labs using a given method. Reproducibility was measured as the standard deviation among lab means and repeatability was determined as the average standard deviation among replicates analyzed within each lab.

Results and Discussion

Acid Detergent Fiber

Several factors significantly affected ADF results. When the normality of the acid was checked (it should be between .95 and 1.05 N), ADF values were lower (32.5 vs. 33.0%). This suggests that the normality of non-checked acid is less than acceptable, which would result in higher fiber values. The use of the Fibertec apparatus also resulted in lower ADF (31.4%) compared to the use of crude fiber refluxing apparatus (33.7%). The lower value for the Fibertec may be due to more effective washing of the fibrous residue that is possible with this apparatus. However, it is more likely due to the larger porosity of Fibertec P1 (90-150 mm) or P2 (40-90 mm) crucibles compared to Gooch coarse porosity (40-60 mm) crucibles. This conclusion agrees with the observation that labs using California Buchner funnels (70-85 mm) obtained ADF values that were 1.6 to 3.0% units lower than labs using other vessels.

Labs that reported soaking in water once compared to those soaking 2 or more times had lower ADF values (31.3 to 31.9% vs. 32.8 to 33.5%). Normally, increasing the number of soakings of fibrous residues reduces fiber values because more detergent and soluble residue is removed. However, the opposite effect can occur with ADF because the ineffective removal of acid during washing can result in loss of fiber during drying of the residues. If all of the sulfuric acid is not removed during washing, it is concentrated on the fiber during drying in the oven because water evaporates more quickly than the acid. Residual acid is drawn to the edges of fiber residues during drying and the combination of concentrated sulfuric acid and high temperature in the drying oven causes a charring of the fiber. This represents a loss of organic matter from the ADF residue resulting in low ADF values.

Neutral Detergent Fiber

It was difficult to detect differences among specific changes in technique because few laboratories reported using the same modification of the NDF method. However, differences among filtering vessels were observed to affect NDF results. Fibertec P2 crucibles (41.8%) and Gooch crucibles with microfibre filter mats (42.5%) obtained lower NDF than Gooch crucibles (43.4%), filter paper in Buchner funnels (43.7%) and unspecified "other vessels" (43.9%). The lower value for Fibertec P2 crucibles may be related to their larger pore size. The result using Gooch crucibles with microfibre filter mats is less clear because these vessels have the smallest particle retention size of all those used. Interactions between method modifications and filtering vessel may have confounded our ability to make comparisons.

Major modifications of the original NDF procedure include changes in sample size and the use of amylase and sodium sulfite. Three methods used by at least eleven labs were evaluated: (1) 1.0 g sample refluxed in 100 mL ND solution with amylase but no sodium sulfite, (2) 0.5 g sample refluxed in 100 mL ND solution with amylase but no sodium sulfite, and (3) 0.5 g sample refluxed in 50 mL ND solution with amylase and sodium sulfite. The small repeatability error for each modification (about 1% of the mean) indicates that each laboratory performed its NDF method precisely (Table 1). The reproducibility of these methods was three to four times that expected of an AOAC approved method, suggesting the minor modifications within and among methods are creating differences among labs. The use of sulfite resulted in significantly lower NDF than when it was excluded.

Conclusions

Numerous modifications of fiber methods were reported by participating labs which resulted in differences among laboratories. It appears that checking the normality of acid detergent solution and standardizing the apparatus, filtering vessel, and number of soakings during the residue washing are critical in obtaining ADF results that compare with other laboratories. Type of filtering vessel and the use of sodium sulfite significantly affect NDF results among labs. It was concluded that in-house methods or modifications of standard methods are major sources of variation in fiber analyses among forage testing laboratories that must be addressed before reproducibility among labs can be improved.

Table 1. Effect of modifications in the NDF method on results.

Modification	Average	Reproductibility[1]	Repeatability[2]
1.0 g sample/100 ml, amylase, no sulfite	46.0	1.9	.37
.5 g sample/100 ml, amylase, no sulfite	45.4	1.5	.45
.5 g sample/100 ml, amylase and sulfite	42.9	1.4	.50
All three modifications	44.5	1.9	.47

[1]Reproductibility among labs = standard deviation among labs.

[2]Repeatability within labs = standard deviation among replicate analyses within labs.

Sources of Variation in Routine Forage Analyses Affecting Dry Matter and Crude Protein Determinations

D.R. Mertens

Introduction

Many factors cause variation in analyses within and among laboratories that result in inaccurate feed evaluation. The greatest source of error (variation) among laboratories is probably associated with differences in methods. Variation among laboratories occurs because methods are often poorly written and difficult to follow by technicians, instructions are often passed from one technician to the next during training resulting in gradual modification of the original method, and methods are often changed to suit the operation of the lab. These changes in methods, whether unintentional or not, often result in differences (biases) among labs that erode confidence in the value of feed analysis.

Materials and Methods

A questionnaire used by the National Forage Testing Association (NFTA) in 1993 contained questions about dry matter (DM) and crude protein (CP). Of the approximately 150 labs participating in the NFTA certification program for 1993, approximately 100 provided information about DM methods and 120 provided information about CP methods. Because answers were not always provided for every question, the data sets actually available for analyzing the effects of individual responses or combinations of responses on analytical results were often less than these maximum numbers of observations.

Because data sets were incomplete, the General Linear Models procedure of SAS was used to statistically analyze the results of the questionnaire. Initially, the analytical value for a method was compared to all question responses for that method. Non-significant responses were removed from the statistical analyses until a final set of responses remained that had a significant impact on results. The analyses provided in this paper are preliminary and represent only the most significant factors affecting results among laboratories. Follow-up responses will be obtained from some labs to create a more complete data set and permit a more thorough analysis of factors causing the differences in analytical results among laboratories.

Results and Discussion

Dry Matter

Of the factors affecting DM analyses, time and temperature of drying had the greatest impact. Twenty-one different temperatures (ranging from 57 to 140°C) and sixteen different times (ranging from 2 to 48 hours) were used for DM analyses, giving a total of 47 different combinations. The AOAC method for DM measurement uses 135°C for 2 h. If the rule of thumb that each 10°C decrease in temperature halves the reaction rate is true for DM determination, the AOAC method should be equal to 125°C for 4 h, 115°C for 8 h, 105°C for 16 h, or 100°C for 22 h. The results of the questionnaire indicate that the same relationship between time and temperature is true for measuring DM. The average DM (adjusted for other significant variables) reported by labs was: 92.9% for 2 h at 135°C, 92.9% for 2 h at 130°C, 93.2% for 16 h at 105°C, 92.9% for 16 h at 100°C, and 92.8% for 24 h at 100°C. Most other combinations of time and temperature were significantly different from the AOAC method for DM.

Dry matters determined in porcelain crucibles or AOAC aluminum pans were significantly lower (0.8 to 1.5% units) than those measured in aluminum foil pans or other vessels. Weighing technique significantly affected DM measurement, but the relationship of desiccator use to DM results was not clear. Hot-weighing yielded lower DM than weighing < 10 or 10-20 samples from desiccators in which the desiccant was replaced after 10 to 30 uses. However, hot-weighing was similar to DM determined: (1) when < 10 samples were weighed from desiccators in which desiccant was replaced

after < 10 uses or > 30 uses, (2) when 10-20 samples were weighed from desiccators in which the desiccant was replaced after < 10 uses, or (3) when > 20 samples were weighed from desiccators in which the desiccant was replaced after > 30 uses.

Two-step drying typically resulted in DM that was 1.4% units higher than single-step drying. This significant difference may be due to the absorption of moisture by the sample between the two drying steps. Errors can occur in DM if the sample is not allowed to equilibrate with lab humidity before the first dry weight is taken.

Crude Protein

The effect of the variation in DM results among labs on both CP and ADF analyses was eliminated by expressing the results on an as-is basis. The major significant factor affecting CP analysis was method. The combustion method yielded significantly higher CP (18.6%) than unspecified "other-methods" (17.9%), macro-block (17.8%), and NIRS (17.7%). All remaining methods were not different from these extremes. The higher CP value for the combustion method suggests that many digestion methods may underestimate true CP content in forages.

There appeared to be higher CP values for digestions using mercury and selenium catalysts. A more thorough analysis of the data is needed to determine if these catalysts are critical to CP analyses. Their negative potential impact on the environment raises questions about any recommendation to use mercury or selenium in routine CP analyses. In some statistical analyses, the percentage recovery of standards was significantly related to CP results. Including standards is recommended as a way for labs to routinely monitor the accuracy of CP analyses.

Conclusions

Although a more detailed statistical analysis of the 1993 NFTA certification questionnaire data may suggest that other variables contribute to the variation in results among laboratories, preliminary analysis indicates that several factors affect DM and CP results. Drying time and temperature, as well as drying vessel, can significantly alter DM results. The method selected and catalyst used during digestion have a significant impact on CP analyses.

Competition Between *Ruminococcus flavefaciens* FD-1 and *Fibrobacter succinogenes* S85 for Cellulose and Cellobiose in Batch and Continuous Culture

Y. Shi, and P.J. Weimer

Introduction

Cellulose is the most abundant component of forage and represents the major source of fermentable energy for ruminal microorganisms. Three species of bacteria — *Fibrobacter succinogenes, Ruminococcus flavefaciens*, and *R. albus* — are generally regarded as the predominant cellulolytic microbes in the rumen. Little is known about the interactions among these species. Recent continuous culture studies have revealed that these species have similar capacities for digestion of crystalline cellulose. However, because these species produce different ratios of fermentation endproducts, differences in the relative activities of each species in vivo could have profound effects on ruminal VFA ratios. We have begun systematic studies of the interaction among the ruminal cellulolytic bacteria by examining binary cultures of *R. flavefaciens* FD-1 and *F. succinogenes* S85 in both batch and continuous modes.

Methods

Cultures were grown at 39°C under CO_2 in a modified Dehority medium that contained cellobiose or microcrystalline cellulose as fermentable carbohydrate. Batch culture was conducted in 158 mL serum vials. Continuous culture was conducted in stirred reactors of 875 mL (cellulose, delivered as a segmented slurry) or 150 mL (cellobiose). Samples were withdrawn for analysis of residual cellulose (determined gravimetrically as NDF), soluble sugars (anthrone assay of culture supernatants), fermentation acids (by HPLC of culture supernatants), and cell mass (estimated from nitrogen in cell pellets). Populations of individual species were estimated using signature membrane fatty acids (tridecanoic acid [13:0] for S85, 13-methyltetradecanoate [i15:0] for FD-1) extracted from cell pellets. Because of the amounts of the particular signature fatty acids in these strains, this method allows detection of FD-1 at levels of > 1% of the population, but detection of S85 at levels of only > 7% of the population.

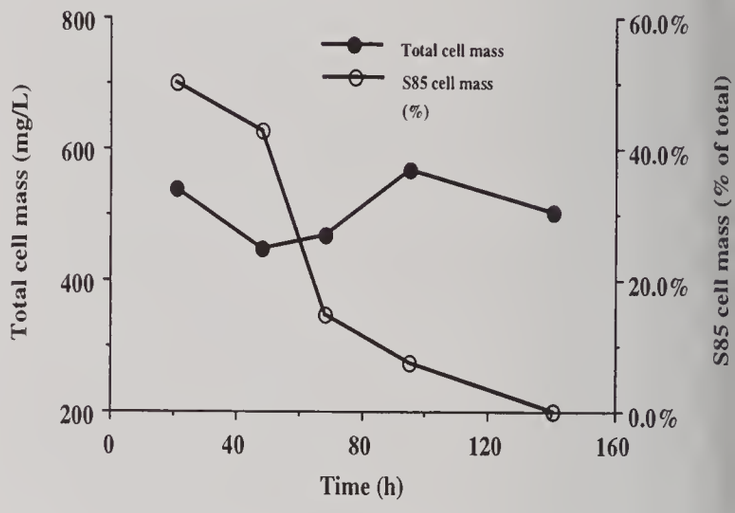


Figure 1. Time course of cellulose-limited continuous culture of R. flavefaciens FD-1 and F. succinogenes S85 when FD-1 was inoculated at 1% by volume to a steady-state culture of S85. Dilution rate = 0.031 h^{-1}, pH 6.5.

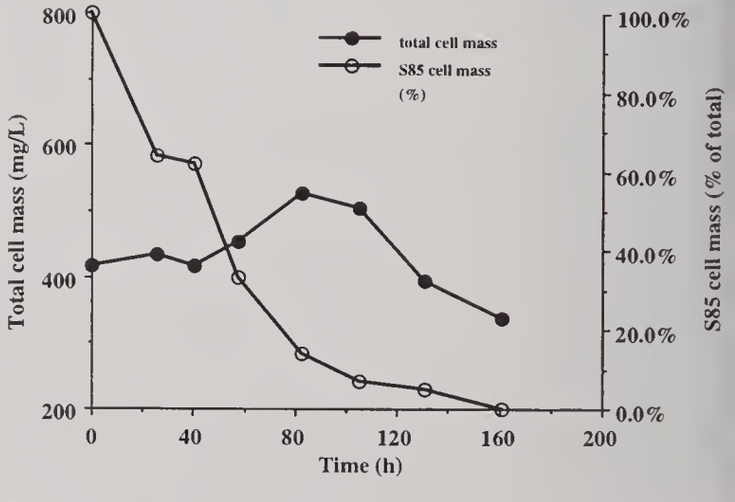


Figure 2. Time course of cellulose-limited continuous culture of R. flavefaciens FD-1 and F. succinogenes S85 when strains were inoculated simultaneously. Dilution rate = 0.031 h^{-1}, pH 6.5.

Results

In batch cultures containing an excess of cellulose and inoculated simultaneously or near-simultaneously, similar populations of both species were present (Table 1). When the inoculation of one strain was delayed until the other strain had fully colonized the available cellulose, the later-inoculated strain accounted for less than 20 per cent of the population. However, if a small amount of cellulose was added to an established S85 culture at the time of inoculation with FD-1, the latter species accounted for nearly half of the population.

In contrast to the batch cultures, continuous cultures fed cellulose as the growth rate-limiting nutrient were rapidly dominated by FD-1, regardless of whether the cultures were inoculated simultaneously (Fig. 1) or FD-1 was added to an established culture of S85 (Fig.2). Because of the limitations of the membrane fatty acid method used, it is not certain if S85 had become eclipsed from the culture. However, the clear dominance of FD-1 over S85 is striking in view of the reputation of S85 as the most intensely cellulolytic ruminal bacterium. Similar dominance was observed within 4 days of inoculating FD-1 into an established, cellobiose-limited continuous culture of S85 ($D = 0.10\ h^{-1}$). In all of the continuous cultures, the amount of cell N was essentially constant throughout each experiment.

Conclusions

Although *Fibrobacter succinogenes* S85 is widely regarded as the most active digester of crystalline cellulose, this strain is readily outcompeted by *Ruminococcus flavefaciens* FD-1 under conditions of cellulose limitation. The coexistence of these species in the rumen may result from the batchwise feeding habits of the ruminant (which results in periods of cellulose excess) and/or the preferential colonization or utilization of different plant cell types.

Table 1. Effect of inoculation pattern on the relative populations of *R. flavefaciens* FD-1 and *F. succinogenes* S85 in 100 mL batch cultures with 300 mg microcrystalline cellulose as sole fermentable substrate.

		% cell mass	
Culture inoculation pattern	Incubation (h)	FD-1	S85
Simultaneous	20	53.0	47.0
S85; 12 min later add FD-1	20	51.8	48.2
FD-1; 10 min later add S85	20	48.4	51.6
FD-1 fully colonized; add S85	40	87.0	13.0
S85 fully colonized; add FD-1	40	18.7	81.3
S85 fully colonized; add FD-1 plus additional 50 mg cellulose	40	42.9	57.1

Comparative Adherence of *Ruminococcus flavefaciens* FD-1 and *Fibrobacter succinogenes* S85 to Microcrystalline Cellulose

Y. Shi, and P.J. Weimer

Introduction

Rapid digestion of cellulose by the most actively cellulolytic ruminal bacteria requires direct contact of these cells to the cellulose. Numerous studies have indicated that pure cultures of ruminal cellulolytic bacteria display differential adherence to cellulose under different environmental conditions. However, few studies have directly compared the adherence kinetics of different cellulolytic species.

Because adherence kinetics are vital in characterizing the interactions among cellulolytic species, we compared the adherence to cellulose of two of the predominant cellulolytic species in the rumen, *Ruminococcus flavefaciens* and *Fibrobacter succinogenes*.

Methods

Pure culture of *R. flavefaciens* FD-1 and *F. succinogenes* S85 were radiolabeled by cultivation in a modified Dehority medium that contained cellobiose (for FD-1) or glucose (for S85) and ^{14}C-2-methylbutyrate. The ^{14}C-labeled cells were washed anaerobically to remove non-incorporated label, and the cell pellets resuspended in media containing 4.8 mg Sigmacell 20 microcrystalline cellulose/mL to a cell density of 0.4-2.5 mg cells/mL. Adherent cells and cellulose were collected by filtration onto 8 µm polycarbonate membranes, and nonadherent cells were collected as the filtrate. Quantitation of both adherent and non-adherent cells was performed by liquid scintillation counting with appropriate corrections for quenching and self-absorption.

Results and Discussion

R. flavefaciens FD-1 adhered to microcrystalline cellulose more rapidly than did *F. succinogenes* S85 (Fig. 1). As has been observed in other studies, adherence appeared to reach a plateau value within a few minutes of exposure of cells to cellulose. Although this plateau value was higher for FD-1 than for S85, a significant fraction of both species did not adhere at the end of the incubation period, despite the fact that the amount of cellulose added contained a surface area in excess of that required to bind the cells. Because the type of cellulose used in this assay is completely colonized by these species during normal growth, it is unlikely that incomplete colonization during the short-term assays is due to a limitation in the number of proper adherence sites for the bacteria. Moreover, adherence of each species was not adversely affected by the presence of unlabeled cells of the other species (Table1), suggesting that adherence sites are in excess and that adherence is limited by some intrinsic property of the organisms. Previous studies by ourselves and others have shown that the fraction of cells capable of adherence is strongly dependent on the age of the culture, suggesting that binding competence is a function of the physiological state of the cells.

The more rapid and more complete adherence of *R. flavefaciens* FD-1 relative to *F. succionogenes* S85 is in accord with our demonstration that the former strain rapidly dominates cellulose-limited continuous cultures inoculated with both strains. Even when FD-1 is added to a well-established axenic culture of S85, the former strain will outcompete S85 for cellulose as it is freshly and continuously fed to the bioreactor. In binary cultures run in a batch mode with excess cellulose, both strains grow to similar densities due to a lack of selective pressure for limited binding sites.

Conclusions

Ruminococcus flavefaciens FD-1 exhibits a greater rate and extent of adherence to crystalline cellulose that does *Fibrobacter succinogenes* S85. This fact undoubtedly contributes to the ability of FD-1 to outcompete S85 in cellulose-limited cultures and may provide a selective advantage to this species in the rumen environment.

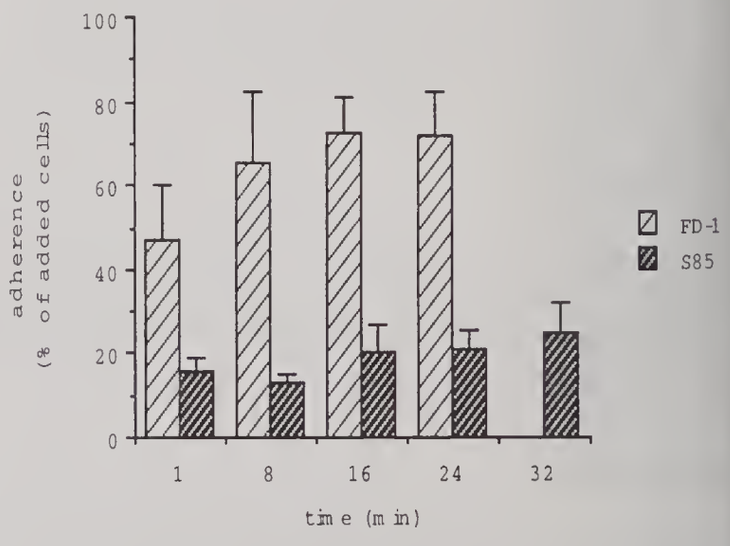


Figure 1. Time course of adherence of R. flavefaciens FD-1 and F. succinogenes S85 to microcrystalline cellulose.

Table 1. Effect of *R. flavefaciens* FD-1 or *F. succinogenes* S85 on the adherence of the other species to microcrystalline cellulose at different incubation times.

Labeled cell	Unlabeled cell	Fraction of labeled cells adhering to cellulose[a]			
		1 min	8 min	16 min	24 min
FD-1	none	41.8	58.4	64.2	74.2
	S85	63.8	70.7	72.5	82.9
S85	none	26.3	23.8	21.7	18.2
	FD-1	30.4	22.4	20.0	29.4

[a]No significant differences ($P > 0.05$) in adherence of each labeled strain in the presence or absence of the other unlabeled strain.

Kinetics of Cellodextrin Utilization by Ruminal Cellulolytic Bacteria

Y. Shi, and P.J. Weimer

Introduction

Cellulose, the major component of forage fiber, is insoluble and is not directly utilizable by ruminal microbes. Instead, it is hydrolyzed by cellulolytic bacteria to soluble oligomers known as cellodextrins. Although both cellulolytic and non-cellulolytic ruminal bacteria have been shown to utilize mixtures of cellodextrins, there have been few studies describing this utilization. Because the outcome of competition among bacteria is largely determined by their ability to utilize available soluble substrates, the kinetics of cellodextrin utilization represents a major potential determinant of competition among ruminal microbes. We therefore examined the utilization of individual cellodextrins by *Fibrobacter succinogenes* and *Ruminococcus flavefaciens*, two of the predominant cellulolytic bacteria in the rumen.

Materials and Methods

Mixed cellodextrins were prepared by treatment of Sigmacell 50 microcrystalline cellulose with fuming hydrochloric acid for 30 min under ice. Excess acid was withdrawn by vacuum and the resulting acid hydroyzate was precipitated five times with acetone. The final precipitate was resuspended in water and lyophilized. Portions (~ 0.4 g) of this cellodextrin mixture were rehydrated in ultrapure water and subjected to preparative scale column chromatography on 50% Celite 545 / 50% Darco G60 saturated with stearic acid. Individual cellodextrins were eluted with a stepwise gradient of ethanol (20%, 30%, 40% by volume). Fractions were assayed colorimetrically by phenol-sulfuric acid; pooled fractions of individual cellodextrins were assayed similarly and their purity verified by HPLC (BioRad 42A column, 85°C, ultrapure water eluant at 0.4 mL/min, refractive index detection). Solutions of pure cellodextrins were heat-sterilized and stored at 4°C prior to use. The cellodextrins included cellotriose (G_3), cellotetraose (G_4), cellopentaose (G_5), and cellohexaose (G_6).

Growth experiments were conducted under CO_2 in anaerobic culture tubes that contained a modified Dehority medium and 0.125 - 1.0 mg/mL concentrations of individual cellodextrins (G_3, G_4, G_5, or G_6), or commercial glucose (G_1) or cellobiose (G_2). Growth tubes were inoculated with 0.2 mL of glucose- or cellobiose- grown *R. flavefaciens* FD-1 or *F. succinogenes* S85 to give a total liquid volume of 5 mL). Cultures were shaken in a vertical position and turbidity readings were recorded at ~0.5 h intervals. To insure constant incubation temperatures, both incubation and turbidimetry were performed in a 39°C room.

Results and Discussion

For both species, plots of the inverse of maximum specific growth rate versus the inverse of substrate concentration for each individual substrate (G_1-G_6) were linear, suggesting that both species displayed standard Monod-type growth kinetics for these substrates. At the low concentrations of cellodextrins added to the cultures, significant efflux of longer chain cellodextrins during growth was not detected. Maximum specific growth rate constants (μ_{max}) and affinity constants (K_s) for the cellodextrins are shown in Table 1. *R. flavefaciens* FD-1 utilized G_2 through G_6, but could not utilize glucose as a growth substrate. This strain exhibited growth rates in the range of 0.25-0.51 h^{-1} and an increase in substrate affinity (lower K_s) with increased chain length up to and including G_5. By contrast, *F. succinogenes* S85 utilized both glucose and higher cellodextrins at maximum growth rates higher than those of *R. flavefaciens* FD-1. However, affinities for these substrates were relatively poor and improved only slightly with increasing chain length.

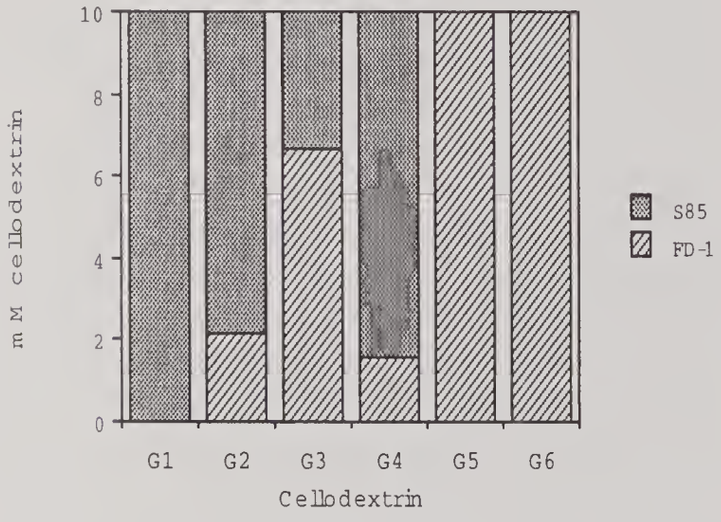


Figure 1. Predicted concentrations of cellodextrins favoring the selective growth of R. flavefaciens FD-1 or F. succinogenes S85 in pure and simple competition.

Application of the kinetic parameters in Table 1 to the Monod equation

$$m = [(\mu_{max})\ (s) / (s + K_s)],$$

where μ = growth rate and s= substrate concentration, reveals that *R. flavefaciens* FD-1, while unable to compete with *F. succinogenes* S85 for glucose, would outcompete this strain for cellodextrins of chain length G_2-G_6 at any physiologically relevant substrate concentration (Fig. 1). The data are in accord with our previous observations that *F. succinogenes* subsists at only low levels (< 7% of the population) in binary culture with FD-1 under conditions of cellulose-limitation. The superior utilization of cellodextrins by FD-1 provides an additional competitive advantage for this strain, in addition to its greater rate and extent of adherence to cellulose.

Conclusions

R. flavefaciens FD-1 is generally superior to *F. succinogenes* S85 with respect to the utilization of soluble products of cellulose hydrolysis. While S85 has a selective advantage over FD-1 with respect to glucose utilization, its low μ_{max} and poor affinity for this substrate suggest that it cannot compete with non-cellulolytic ruminal species for this substrate once outside its protective glycocalyx. The more rapid utilization by both FD-1 and S85 of the longer oligomers at low substrate concentrations suggests that these longer cellodextrins, rather than glucose or cellobiose, are the likely growth substrates for these organisms in the ruminal environment.

Table 1 Kinetic parameters for *R. flavefaciens* FD-1 and *F. succinogenes* S85 on soluble oligomers of cellulose.

		R. flavefaciens FD-1		*F. succinogenes* S85	
Oligomer		μ_{max}(h^{-1})	K_s (mM)	μ_{max}(h^{-1})	K_s (mM)
Glucose	(G_1)	0	—	0.45	0.43
Cellobiose	(G_2)	0.38	0.28	0.41	0.47
Cellotriose	(G_3)	0.47	0.15	0.49	0.44
Cellotetraose	(G_4)	0.43	0.12	0.50	0.40
Cellopentaose	(G_5)	0.39	0.04	0.26	0.33
Cellohexaose	(G_6)	0.35	0.23	0.25	0.18

Binding of Radiolabelled Monensin and Lasalocid to Ruminal Microorganisms and Feed

J.M. Chow, J.S. Van Kessel, and J.B. Russell

Introduction

Monensin has been used to increase the performance of beef cattle and dairy heifers for more than two decades, and lasalocid, a closely related ionophore, has also been approved for use as a ruminant feed additive. Ionophores were originally marketed as enhancers of ruminal propionate production, but subsequent work showed that they could also inhibit lactate- and ammonia-producing ruminal bacteria. Because ionophores inhibited Gram-positive bacteria to a much greater extent than Gram-negative bacteria, it seemed that the Gram-negative outer membrane was able to protect the cell membrane from ionophores. Monensin is typically added to feed at 20 to 30 ppm. Based on a feed intake of 10 kg of DM/d and a ruminal volume of 60 L, the ruminal ionophore concentration would be 5 to 7 μM. In vitro studies often used a similar concentration, but these studies ignored the ability of ionophores to concentrate in bacterial membranes and the fact that the density of microorganisms is usually much greater in vivo than in vitro. Because little was known about the binding of radiolabelled ionophore to ruminal microorganisms and feed particles, we decided to study ionophore binding with radiolabelled monensin and lasalocid.

Materials and Methods

Ruminal contents were removed from a 750 kg nonlactating, ruminally fistulated cow. Protozoa and bacteria were partitioned further by differential centrifugation (117 vs 10,000 x g, 5 min). Outer membrane lipopolysaccharides were disrupted by washing mixed ruminal bacteria twice with 120 mM Tris HCl plus 10 mM EDTA. The final cell concentration of bacteria or protozoa was varied from 0 to 4 mg of protein/mL. Ground alfalfa hay (< 300 μm particle size, 90% DM) was hydrated in phosphate buffer (pH 6.5), and the final concentration was varied from 0 to 60 mg/mL.

Pure cultures of ruminal bacteria were grown anaerobically in medium containing salts, Trypticase, yeast extract, cysteine hydrochloride, and a VFA mixture, and either glucose, cellobiose or Casamino Acids as an energy source. Membrane vesicles of *P. ruminicola* and *S. bovis* JB1 were prepared by osmotic lysis. Mixed bacteria, mixed protozoa, feed, or vesicles were incubated with labeled lasalocid or monensin for 15 min at 39°C. The final concentration of ionophore was 5 μM. The binding of ionophore was estimated from the decrease in radioactivity in supernatant.

Results and Discussion

Gram-negative, ionophore-resistant ruminal bacteria and Gram-positive, ionophore-sensitive species bound similar amounts of radioactive lasalocid, but neither group bound large amounts of radioactive monensin. Membrane vesicles also bound more lasalocid than monensin ($P < .05$). The binding was first order at low cell or vesicle concentrations and saturable at high cell or vesicle densities. *Streptococcus bovis* was inhibited by both monensin and lasalocid (5 μM), but cells that were re-incubated in medium lacking ionophore grew rapidly. Lasalocid-treated cells grew very slowly when they were resuspended in fresh medium. Based on these results, it seemed that lasalocid had a higher affinity for bacterial membranes than monensin. Mixed bacteria, however, bound nearly equal amounts of radioactive monensin and radioactive lasalocid ($P > .05$). Monensin binding was greatly reduced when the mixed ruminal bacteria were pretreated with Tris+EDTA ($P < .05$), but Tris+EDTA did not affect the binding of lasalocid. Mixed ruminal protozoa always took up more lasalocid than monensin ($P < .05$), but feed particles bound equal amounts of lasalocid and monensin ($P > .05$). Based on the binding capacity of mixed ruminal bacteria, ruminal protozoa, and feed particles, there would be little free ionophore in ruminal fluid.

Monensin and lasalocid mediate the exchange of cations for protons across cell membranes. When these metal/proton antiporters bind a cation, the molecule assumes a torus-shape (doughnut), the cation is shielded, and the electroneutral ion/ionophore complex can transverse the membrane. After the cation dissociates, the carboxyl group of the ionophore can bind a proton, and this other electroneutral complex proceeds in the opposite direction. The proton is then released and the cycle continues. This disruption of ion balance across the cell membrane, leads to a loss of intracellular K, an accumulation of intracellular Na, an acidification of the cytoplasm, a loss of transport activity, and a depletion of ATP.

Both monensin and lasalocid are hydrophobic, but it seems that these ionophores differ in their lipid solubility. Lasalocid dissolved less readily in ethanol than monensin, and membrane vesicles of ruminal bacteria took up at least 10-fold more lasalocid than monensin. Because membrane vesicles of *P. ruminicola*, a Gram-negative, ionophore-resistant species, and *S. bovis*, a Gram-positive, ionophore-sensitive species, took up similar amounts of lasalocid and monensin, it did not seem that ionophore resistance could be explained by differences in the cell membrane per se.

Ionophores must pass through outer layers of the cell envelope before they can reach the cell membrane. Gram-negative bacteria are surrounded by an outer membrane, but Gram-positive bacteria lack this cell wall structure. Because *P. ruminicola* whole cells had a higher K_b than the membrane vesicles ($P < .05$), it seemed that the outer membrane of *P. ruminicola* had a lower affinity for ionophore than the cell membrane. Whole cells and membrane vesicles of *S. bovis* JB1, a Gram-positive bacterium, had similar K_b values ($P > .05$).

Conclusion

The potency of antibiotics has often been designated by the minimum inhibitory concentration (MIC), and this term has been used in the assessment of the effect of ruminal ionophores. The extrapolation of in vitro experiments to in vivo conditions can, however, present problems in interpretation. The total count of bacteria in the rumen is usually 10^{10} cells/mL or greater, but the density of bacteria in vitro is usually 10-fold less (10^8 o 10^9/mL). Because the ratio of ionophore:bacterial mass appears to be a more important criterion of ionophore potency than the absolute concentration per se, much of the early in vitro work on ruminal ionophores was performed at high and unphysiological concentrations.

Alternative Strategies of Low Affinity Glucose Transport in Ruminal Bacteria

G.M. Cook, and J.B. Russell

Introduction

The phosphoenolpyruvate (PEP): phosphotransferase system (PTS) of group translocation is widely distributed in fermentative bacteria, but relatively few ruminal bacteria have PTS activity. *Streptococcus bovis* and *Selenomonas ruminantium* have PTS systems for glucose and disaccharides, and these bacteria can ferment sugars at a faster rate than other ruminal microorganisms. The rapid conversion of sugar to lactic acid can cause a decrease in ruminal pH, an inhibition of cellulose digestion, founder and even death of the animal.

The mannose PTS of *E. coli* phosphorylates the nonmetabolizable glucose analog, 2-deoxyglucose (2-DG), and 2-DG has been used as a tool for demonstrating glucose PTS activity. The active transport of 2-DG does not produce 2-DG-phosphate because bacterial glucokinases cannot generally recognize 2-DG, and this difference in 2-DG specificity has also been used as a method of selecting PTS-deficient mutants. 2-DG-6-phosphate arising from the the PTS is dephosphorylated by 2-DG phosphatase, and the cycle of 2-DG phosphorylation and dephosphorylation depletes the cell of PEP and ultimately ATP.

Materials and Methods

S. ruminantium HD4 and *S. bovis* JB1 were grown anaerobically at 39° C in a medium containing salts, yeast extract, trypticase, cysteine hydrochloride and volatile fatty acids. 2-DG resistant cells were selected with 5 mM glucose and 20 mM 2-DG. Cells were harvested during exponential growth by centrifugation and washed twice in 100 mM Na/K phosphate buffer containing 5 mM MgCl2 and 2 mM dithiothreitol. Cells were then resuspended in 2 ml of the same buffer and stored on ice. The cell suspension was treated with toluene:ethanol mixture. PTS and glucokinase activity was determined in toluene-treated cells at 39° C by enzymatic methods. Cells were incubated anaerobically with radiolabelled sugar for 10 sec. Transport was terminated by the addition of 2 ml ice-cold 0.1 M.

Results and Discussion

Streptococcus bovis and *Selenomonas ruminantium* grew in the presence of the glucose analog, 2-deoxyglucose (2-DG), but the cells no longer had high affinity glucose transport. In *S. bovis*, 2-DG resistance was correlated with a decrease in phosphoenolpyruvate (PEP)-dependent glucose phosphotransferase (PTS) activity. The 2-DG-selected *S. bovis* cells relied solely upon a low affinity, facilitated diffusion mechanism of glucose transport and a 2-DG-resistant glucokinase (ATP-dependent). The glucokinase activity of *S. ruminantium* was competitively inhibited by 2-DG, and the 2-DG selected cells continued to use PEP-dependent PTS as a mechanism of glucose transport. In this latter case, the 2-DG selected cells switched from a mannosephosphotransferase (enzyme II) that phosphorylated glucose, mannose, and 2-DG, but not α-methylglucoside to a glucosephosphotransferase (enzyme II) that phosphorylated glucose and α-methylglucoside but not 2-DG or mannose. The glucosephosphotransferase (enzyme II) had a very low affinity for glucose and the transport kinetics were similar to the facilitated diffusion system of *S. bovis*.

In nature, sugars are often found at very low concentrations, and bacterial competition for these energy sources can be very intense. Many bacteria have evolved very efficient systems for translocating of sugars, but bacterial membranes are normally "crowded" with pumps, carriers and channels. When sugars are present at high concentrations, affinity is no longer the rate limiting step in substrate utilization and success is dependent on the maximum rate of uptake. Because channels have a much higher turnover number than carriers, facilitated diffusion could offer a more rapid means of sugar utilization when sugar concentrations are high.

Conclusion

Based on our results, it appears that *S. bovis* and *S. ruminantium* have markedly different strategies of 2-DG resistance. In *S. bovis*, 2-DG resistance is mediated by a general loss of PTS activity, and a reliance on low affinity, facilitated diffusion as an alternative mechanism of glucose transport. The facilitated diffusion system transports both glucose and 2-DG [16], but the glucokinase of *S. bovis* does not phosphorylate 2-DG and is not inhibited by 2-DG. Because the *S. ruminantium* glucokinase is strongly inhibited by 2-DG, facilitated diffusion is not a suitable mechanism of transporting glucose when 2-DG is also present. *S. ruminantium*, however, appears to have two separate PTS systems for glucose, and the glucose (glucosephosphotransferase enzyme II) PTS is not able to take up 2-DG. By switching from the mannose PTS to a low-affinity glucose PTS, *S. ruminantium* excludes 2-DG from the cell and protects its 2-DG-sensitive glucokinase. The glucose PTS has a much lower affinity for glucose than the mannose PTS, and this difference in affinity explains the biphasic kinetics of glucose uptake by *S. ruminantium*.

The Effect of Extracellular pH and Lactic Acid on pH Homeostasis in *Lactococcus Lactis* and *Streptococcus Bovis*

G.M. Cook, and J.B. Russell

Introduction

For many years, it was assumed that bacteria had a more or less constant intracellular pH over even wide ranges of external pH. The pH regulation of *Escherichia coli* has been studied in great detail, and this bacterium does seem to regulate its intracellular pH within a rather narrow range. Recent work, however, indicated that some fermentative bacteria let their intracellular pH decrease as a function of extracellular pH.

Glycolyzing and growing *Lactococcus (Streptococcus) lactis* cells were reported to have intracellular pH values greater than 7.0, even if the extracellular pH was as low as 5.0. The ruminal bacterium, *Streptococcus bovis*, grew at pH values as low as 4.7, and at this external pH, the intracellular pH was only 5.4. Based on these comparisons, the question then arose, why do these lactic-producing bacteria appear to have such strikingly different strategies of intracellular pH regulation?

Materials and Methods

Streptococcus bovis JB1 and *L. lactis* ML3 were grown anaerobically at 39° C in MRS medium at 39° C. The pH gradient across the cell membrane (ΔpH) and the electrical potential ($\Delta\Psi$) were determined by methods employing silicon oil centrifugation and radiolabeled benzoate and TPP. Intracellular volumes were estimated from the difference between radiolabeled PEG and water. ATP was assayed with a luminometer. Exponentially growing cultures were centrifuged through silicon oil and the cell pellets were digested with nitric acid. The potassium concentration of the cells was then determined by flame photometry. The specific rate of glucose consumption (i.e. enthalpy, 21 kcal /mmole lactate produced) was measured with an LKB model 2277 Bioactivity monitor.

Results and Discussion

When *Streptococcus bovis* JB1 and *Lactococcus lactis* ML3 were grown with an excess of glucose, lactic acid accumulation caused a decrease in extracellular pH. *S. bovis* grew at extracellular pH values as low as 4.9, but *L. lactis* was unable to grow below pH 5.3. Because both bacteria maintained a low ΔpH across the cell membrane, it appeared that intracellular pH was controlling their pH sensitivities. *S. bovis* glycolyzed glucose and maintained high concentrations of ATP at intracellular pH values as low as 5.4. *L. lactis* could not glycolyze glucose when the intracellular pH was less than 5.6, and ATP declined. *L. lactis* cells which were washed and incubated in buffers with an excess glucose had higher ΔpH values. Lactic acid addition, however, prevented the interconversion of membrane potential ($\Delta\Psi$) and chemical gradient of protons ($Z\Delta$pH).

It has long been recognized that many bacteria cannot grow in the presence of fermentation acids at low pH. The toxicity of fermentation acids at low pH was often explained by metabolic uncoupling, but recent work indicated that there was little basis for this hypothesis. Synthetic uncouplers are lipid soluble in both the protonated and unprotonated state, and their ability to cycle protons through the cell membrane can cause a rapid decline in protonmotive force. Fermentation acids also transverse the cell membrane in the protonated state, but the anions are much less permeable. The impermeability of fermentation acid anions does not permit a continued flux of protons through the cell membrane.

The accumulation of lipid permeable acids (e.g. benzoate or salicylate) has provided a method of estimating the intracellular pH of bacteria, but the importance of fermentation acid anion accumulation

in bacteria was largely ignored. As the pH gradient (ΔpH) across the cell membrane increases, lipid permeable acids transverse the cell membrane and dissociate in the more alkaline interior. If the anion is impermeable, the concentration of anion will increase in accordance with the Henderson-Hasselbalch equation. Because the relationship between ΔpH and anion accumulation is logarithmic, an even modest increase in ΔpH can cause a dramatic increase in the concentration of intracellular fermentation anions.

In this study, *S. bovis* grew until the extracellular pH was less than 5.0, and the ΔpH at this point was only 0.7 units. The glycolytic rate decreased as the intracellular pH declined, but neither ATP nor intracellular potassium decreased. Therefore, it did not appear that metabolic energy per se was the critical aspect of the pH sensitivity. Based on these results, it appeared that biosynthesis (e.g. protein synthesis) was the more pH sensitive aspect of growth. *L. lactis* also decreased its intracellular pH and glycolytic rate when the external pH declined, but in this case there was a large decrease in intracellular ATP. Since ATP declined, it appeared that energy availability for growth was, in this case, a key factor in the pH sensitivity.

Other workers indicated that *L. lactis* had a ΔpH of approximately 2.0 units at an extracellular pH of 5.0, but these experiments were performed with glycolyzing, non-growing cells in a buffer which did not contain lactate. When we performed similar short term experiments with *L. lactis*, ΔpH increased as the extracellular pH declined, but there was no increase in ΔpH if the pH of the incubation buffer was adjusted downward with lactic acid (30 mM or less) rather than HCl. *S. bovis* always had a low ΔpH (even if lactate was not added), but it should be noted that this bacterium had a much higher initial rate of lactate production.

Conclusion

Based on these results, it appears that fermentative bacteria like *L. lactis* and *S. bovis* must decrease their intracellular pH and maintain a low ΔpH across the cell membrane. High ΔpH would cause a large and toxic accumulation of fermentation acid anions. The decrease in intracellular pH, however, presents a potential constraint to various aspects of bacterial growth (ATP production, transport, biosynthetic reactions, non-growth ATP utilization, etc.). The critical aspect of a particular bacterium's pH sensitivity may vary.

Preserved Mixed Ruminal Microbes as Inoculum Source for In Vitro Determination of Protein Degradation

N.D. Luchini, G.A. Broderick, and D.K. Combs

Introduction

Mixed ruminal microorganisms (MRM) are used commonly for in vitro determination of ruminal protein degradation. Our group has applied an inhibitor in vitro (IIV) procedure (Broderick, 1987) to estimate rate and extent of ruminal protein degradation. Among the limitations of the IIV method are: 1) variation in inoculum activity, 2) inaccuracies due to high levels of NH_3 and total amino acids (TAA) in blanks, and 3) the need for a rumen donor cow. Commercial proteases have proven unsatisfactory for estimating ruminal degradation because their activities do not mimic those of MRM. A method for preserving MRM for use as an inoculum for in vitro determination of ruminal protein degradation was developed. Despite ranking proteins similarly for degradability, inocula made from preserved MRM yielded lower rates and extents of degradation than inocula made with fresh MRM. Two experiments were conducted to evaluate methods of preserving MRM and effects of preincubation of preserved MRM on protein degradation rate.

Materials and Methods

Whole ruminal contents were obtained from a ruminally cannulated lactating dairy cow 2 h after feeding. Strained ruminal fluid (SRF) was produced by squeezing contents through two layers of cheesecloth; remaining solids were washed 4 times with a total volume of 39°C McDougall's buffer equal to the original volume of SRF. The SRF plus buffer wash were mixed, filtered through cheesecloth, centrifuged (5,000 x g, 30 min, 4°C), and the supernatant discarded. In Trial 1, the pellet was resuspended in a 50:50 glycerol:McDougall's buffer and stored at -20°C; 120 ml of glycerol:buffer solution was used to resuspend the MRM pellet from 2.4 L of SRF plus buffer. In Trial 2, MRM was prepared as described for Trial 1 [except stored at -80°C instead of -20°C (Frozen MRM)] or glycerol was added at 5% of the volume of SRF plus buffer, stirred for 30 min, centrifuged as in Trial 1, and the pellet frozen and lyophilized (Lyophilized MRM).

In Trial 1, MRM glycerol:buffer suspension was thawed at room temperature and reconstituted to a volume equivalent to the discarded supernatant using 39°C media consisting of macro- plus micromineral buffer with added vitamins, VFA, hemin and carbohydrates (Hristov and Broderick, 1994). Reconstituted MRM was preincubated for 6 h at 39°C under O_2-free CO_2; concentrations of NH_3 and TAA were monitored. Rate and extent of protein degradation for expeller (ESBM) and solvent soybean meal (SSBM) were determined by IIV using inocula prepared from MRM preincubated 6 h and MRM that was not preincubated. In Trial 2, inocula prepared from Frozen MRM, Lyophilized MRM, and Fresh MRM were preincubated for 24 h at 39°C; changes in gas production, VFA, OD, NH3, and TAA were monitored. A set of inocula were prepared from Frozen MRM and Lyophilized MRM (preincubated for 16 h at 39°C) and Fresh MRM (dialyzed 2 h against saline to lower background NH_3) to determine rate and extent of protein degradation of ESBM and SSBM.

Results and Discussion

Preincubation of MRM substantially reduced blank concentrations of NH_3 and TAA at both 0 and 4 h ($P < .005$; Table 1), suggesting that this was effective for depleting background levels of protein degradation products. Degradation rates for ESBM and SSBM also were higher ($P < .01$; Table 1) for the preincubated inoculum, indicating a net increase in microbial activity occurred during preincubation. Degradation rates obtained with preincubated inoculum were more similar to our long-term IIV means of .037 and .141/h for ESBM and SSBM, respectively. Thus, preincubation improved the estimates of rate and extent of protein degradation obtained with MRM stored frozen.

Although it had a brief lag relative to Fresh MRM, Frozen MRM tended to have higher rates of gas production than the other two inocula. Greater gas production in Frozen than Fresh MRM may be explained by the glycerol added to Frozen MRM. A lag time observed with Lyophilized MRM prior to significant gas accumulation suggested about 2 h was required for Lyophilized MRM to rehydrate and equilibrate with the medium. Patterns of production of VFA, without net lactate accumulation, during the 24 h preincubation with Fresh MRM indicated a normal fermentation. The VFA patterns of Frozen MRM and Lyophilized MRM indicated that, despite possible early shifts in the microbial population, the only difference from Fresh MRM was a somewhat elevated propionate concentration after 6 to 8 h of preincubation. Levels of VFA were within the normal range for in vivo concentrations. Measurements of OD during preincubation indicated that both Fresh and Lyophilized MRM showed a lag time, then exponential growth, followed by a stationary phase. Frozen MRM showed linear growth throughout the 24 h preincubation. The pH was very stable during preincubation of all three inocula. Concentration of NH_3 in Fresh MRM was higher than Frozen and Lyophilized MRM throughout the 24 h preincubation. Concentrations of both NH_3 and TAA were lowest in Frozen

MRM. Low concentrations of N substrates in Frozen MRM may have limited microbial growth. Rates of ruminal degradation of ESBM and SSBM proteins were computed from net (i.e., blank corrected) release of NH_3 and TAA. Degradation rates for ESBM were .013, .012, and .003/h, and for SSBM were .081, .075, and .017/h, for Fresh, Frozen, and Lyophilized MRM, respectively. Rates for Fresh and Frozen MRM were more rapid ($P < .01$) than for Lyophilized MRM indicating that Lyophilized MRM did not have degradative activity similar to Fresh or Frozen MRM, even after 18 h of preincubation.

Conclusions

Preincubation of reconstituted MRM, previously preserved frozen, both reduced NH_3 and TAA background levels and improved protein degradative activity. Fermentation characteristics of reconstituted inocula suggested that there were no major shifts in microbial population and inocula were stable over a 24 h preincubation. This would permit preincubation to be carried out overnight. Degradative activity toward ESBM and SSBM of inocula made with Frozen MRM (preincubated for 18 h) and dialyzed Fresh MRM were similar and greater than that of inocula made with Lyophilized MRM. The MRM may be preserved frozen and, after preincubation, can be used for in vitro determination of ruminal protein degradation.

Table 1. Blank concentrations of NH_3 and total amino acids (TAA) and protein degradation rates[1] of expeller soybean meal (ESBM) and solvent soybean meal (SSBM) in incubations with mixed rumen microorganisms stored at -20°C and preincubated for 0 or 6 h.

	Blank concentrations (mM)				Degradation rates (/h)	
Preincubation	0 h		4 h			
time (h)	NH_3	TAA	NH_3	TAA	ESBM	SSBM
0	1.00	.90	2.06	2.87	.011	.023
6	.64	.24	1.07	.42	.018	.099
P <	.005	.001	.001	.001	.010	.010

[1]Degradation rates estimated from net release of NH_3 and TAA after 0 and 4 h of in vitro incubation.

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Characteristics of Alfalfa Hay Quality Grades Evaluated by the Relative Feed Value Index

C.S. Kuehn, H.G. Jung, J.G. Linn, and N.P. Martin

Introduction

Alfalfa hay is marketed in the upper Midwest in a system with six quality grades — prime and standards one through five. Hay is assigned to these quality grades based on the relative feed value (RFV) index which is calculated from the neutral detergent (NDF) and acid detergent (ADF) fiber content of the forage. As part of a biomass energy feasibility study conducted with Northern States Power Company and the University of Minnesota, we sought to determine the botanical and chemical composition of commercial alfalfa hay sold in Minnesota. Our objective was to determine if the alfalfa leaf fraction in commercial hay was variable in nutrient composition or relatively consistent in quality as seen for agronomic plot research. Quality of the alfalfa leaves is critical to the financial success of an alfalfa biomass energy system.

Material and Methods

Alfalfa hay samples were collected from four commercial hay auctions in Minnesota. Prior to sale, hay lots were sampled and analyzed by near-infrared reflectance spectroscopy. From this analysis, RFV was calculated and hay was assigned to a quality grade. One representative small square bale was purchased from nine prime, 12 standard one, nine standard two, six standard three, one standard four and one standard five hay lots. Representative samples of each bale were manually separated into coarse stems and weeds, mostly grasses. Remaining leaves and small stems were separated with a mechanical sieve. The screen sizes were 190.5, 127, 63.5, 39.6 and 11.7 mm square, with thickness varying from 127 to 6.4 mm. Stem material was considered to be all material that did not pass through the 63.5 mm screen. This material and the coarse stems from the initial sorting process were composited as the total stem material. Leaf material was considered to be all the remaining material which did not pass through the 11.7 mm screen. Samples were ground in a Wiley mill through a 1-mm screen. Stem, leaf, and weed components were analyzed for crude protein (CP), NDF and ADF.

Results

The results of the NDF and ADF analyses were used to calculate an actual RFV index for each sampled hay bale. The leaf to stem ratio for the alfalfa portion of the hays was significantly different among the quality grade samples, but there was only a poor trend toward more leafiness of higher quality hays (Table 1). While there were large differences among the quality grades for percentage weeds, these differences were not significant because of extreme variability within each individual set of quality grade samples. Twenty-eight of the hay bales contained less than 10% weeds and were used for analysis of alfalfa leaf nutrient composition (Table 2). Alfalfa leaf CP concentration did not vary significantly among the hay quality grades, although there was a trend toward reduced CP in the lower quality grades. This reduction in leaf CP in poor quality grades may be real as evidenced by the significant increase in NDF content of the lower quality grades. Alfalfa stems showed a very consistent reduction in quality with lower grades for both CP and NDF.

Conclusion

The data indicate that some low RFV hay is actually quite leafy alfalfa, but the presence of large amounts of grasses and weeds depress the overall quality grade of the hay. The reduction in leaf CP and elevated NDF of lower quality grade hay are probably a reflection of leaf deterioration during field drying. Poor stem quality will primarily result from delayed harvested and advanced maturity of

the crop. The presence of weeds and low alfalfa leaf to stem ratios will severely reduce the yield of alfalfa leaves from a biomass crop. This depression in leaf yield coupled with reduced leaf nutrient quality will adversely affect the economics of alfalfa hay as a biomass energy crop. These issues must be addressed in the development of an alfalfa based biomass energy system.

Table 1. Average RFV, leaf to stem ration, and weed content of hays within quality grades.

Grade	N	RFV	Leaf/Stem	Weeds (% DM)
Prime	5	179	1.7[a]	2.4
One	6	140	1.0[bc]	2.6
Two	9	108	1.3[ab]	12.1
Three	12	94	0.8[c]	13.8
Four	3	80	1.4[ab]	34.4
Five	4	66	0.6[c]	36.5

[abc]Means in the same column not sharing a superscript are different ($P < 0.05$).

Table 2. Chemical composition of the leaf and stem fractions from alfalfa hay bales containing less than 10% weeds.

	Leaves		Stems	
Grade	CP	NDF	CP	NDF
	- - - - - - - - - - - - - - % DM - - - - - - - - - - - - - - -			
Prime	26.0	26.2[a]	12.8[ab]	52.1[a]
One	25.4	31.5[b]	13.3[a]	56.3[b]
Two	25.9	39.6[c]	12.3[b]	67.6[c]
Three	24.8	39.6[c]	11.3[b]	68.5[c]
Four	nd	nd	nd	nd
Five	22.7	48.4[d]	9.1[c]	76.1[d]

nd - Not determined.

[abcd]Means in the same column not sharing a superscript are different ($P < 0.05$).

Economic Value of Alfalfa Leaf Meal as a Protein Supplement for Lactating Dairy Cows

H.G. Jung, J.G. Linn, and C.S. Kuehn

Introduction

The economic and engineering feasibility of a biomass energy system for electricity production was studied in cooperation with Northern States Power Company and the University of Minnesota. Alfalfa was chosen as the biomass crop for study because it offered the potential for revenue both from power generation and sale of a valuable feed co-product. The concept is to produce alfalfa hay and then fractionate it into stem material for conversion to electricity and a leaf meal product for use

as a protein supplement for livestock. Because of the chemical composition of alfalfa leaves, dairy cattle are the most likely consumers of such a leaf meal product. This report summarizes our investigation of the possible inclusion rates and economic value of alfalfa leaves for lactating dairy cattle.

Materials and Methods

Least-cost rations were formulated to meet NRC requirements for cows in early (90 lb/d) and mid lactation (60 lb/d). Feedstuffs typically used in upper midwest dairy rations were available for inclusion in the rations. Summer 1994 prices were used for feeds (Table 1), except for corn grain and soybean meal which were included at high ($2.50/bu & $200/T, respectively) and low ($2.00/bu & $160/T, respectively) prices. Alfalfa leaf meal nutrient content was estimated as the average chemical composition determined on the leaf fraction obtained from an experimental separation of commercial alfalfa hay bales purchased from 27 lots at Minnesota auctions in 1993/94. The potential price for alfalfa leaf meal was determined from other feed prices and usage in rations. All rations included 5 lb alfalfa hay/cow/d.

Results and Discussion

Alfalfa leaf meal averaged 25.2% CP, 6.3% ADIN, 36.0% NDF, 21.5% ADF, 5.3% ADL, 2.9% ether extract, 11.3% ash, 1.88% Ca, 0.33% P, and had an IVDMD of 73.5%. The NEL of the alfalfa leaf meal was estimated to be 0.69 Mcal/lb. This leaf meal obviously contained some stem material based on both visual observation and published data which indicates alfalfa leaves contain 30% CP, 20% NDF, 15% ADF, 2.5% Ca, and 0.27% P. Efficiency of the separation of leaf from stem has major economic effects on the value of the leaf meal product, as will be demonstrated later, and also has a tremendous impact on the overall economic viability of the alfalfa biomass energy system.

For a 1350 lb cow producing 3.8% fat milk, formulation of a high alfalfa silage vs. corn silage ration has important implications for the level of inclusion of alfalfa leaf meal (Table 1). For cows producing 60 lb/d of milk and fed a high alfalfa silage diet there would be no inclusion of alfalfa leaf meal in the ration because the major feed ingredients could supply all of the required protein. Because of the low protein content of corn silage compared to haylage, on the high corn silage ration up to 10.5 lb of alfalfa leaf meal could be fed daily when soybean meal is not an economic alternative protein source. For the high producing cow (90 lb milk/d), some alfalfa leaf meal was included in the high haylage diet. This is because early lactation cows need more protein but cannot consume as much hay and haylage because of their high energy requirements. However, on the high corn silage diet less leaf meal was included in the early lactation cow diet than for the mid lactation cow because the moderate energy content of the alfalfa leaf meal limited its inclusion into the diet. The other potential protein supplements would be included in the diets because they provide protein in combination with higher energy values.

Table 2 shows the range in possible value of alfalfa leaf meal for dairy cattle. The leaf meal could sell for as little as $93.88 or as high as $108.84 per ton depending on corn and soybean meal prices and dietary inclusion rate. The prices shown are the maximum prices that a farmer should consider paying for alfalfa leaf meal given the nutrient composition assumed in this study and the prices used for the other feed ingredients. Increasing the purity of the leaf separation to yield a higher CP (30%) and energy (0.77 Mcal NEL/lb) feed would increase the economic value of the leaf meal in our example by about 15%.

Conclusion

The livestock industries have been open to incorporating non-traditional feeds into their animal rations if a few important requirements are satisfied. Acceptance of alfalfa leaf meal as a feedstuff by dairy producers will depend on year-round availability of the leaf meal, a large volume of this feedstuff, and a consistent leaf meal quality. The biomass energy project has the potential to satisfy these requirements.

Table 1. Inclusion rates of alfalfa leaf meal in rations with different amounts of haylage or corn silage.

Feed Ingredient	Milk Production 60 lb/d		90 lb/d		Feed Price
	lb/cow/d				$/T
Hay	5.0	5.0	5.0	5.0	120
Haylage	40.0	14.1	29.3	12.5	45
Corn Silage	15.0	42.4	15.0	40.0	22
Corn	15.7	8.5	19.7	13.6	Variable
Fat	0	0	0.3	0.4	360
Vitamin & Mineral	0.6	0.6	0.9	0.9	440
Soybean Meal	0	0	3.1	4.3	Variable
Cottonseed	0	0	5.0	3.6	180
Distillers Grains	0	0	3.1	4.3	145
Alfalfa Leaf Meal	0	10.5	3.2	7.6	Floating

Table 2. Economic value of alfalfa leaf meal in rations balanced for either 60 or 90 pounds of milk per day with variable corn and soybean meal prices.

	Soybean meal, $/ton	160.00	160.00	200.00	200.00
Milk, lb/day	Corn, $/bushel	2.00	2.50	2.00	2.50
		$/ton			
60		96.64	94.07	108.84	106.26
90		93.88	95.18	102.87	104.17

Heat Treatment of Alfalfa Leaf Meal for Improved Protein Degradability

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Introduction

The economic and engineering feasibility of a biomass energy system for electricity production using alfalfa hay as the biomass crop was studied in cooperation with Northern States Power Company and the University of Minnesota. It was found that the value of a leaf meal co-product for use as a dairy cow protein supplement was critical to the economic viability of the biomass energy project. Because of the very low price of coal (~$20/T) and the lower energy content of biomass (~75% of coal), the stem fraction of the alfalfa only has a real economic value of about $15/T. If this biomass energy system is to be viable without some form of subsidy, then the leaf meal product must have as great a value as possible.

One possible way to increase the feeding value of the leaf meal would be to increase its undegradable intake protein (UIP) fraction. Previous work at the USDFRC has shown that heating of whole alfalfa increases its UIP content. Because an electric power plant has waste heat available, we chose to examine the responsiveness of alfalfa leaf meal to heating both for UIP content and intestinal availability.

Materials and Methods

A composite sample of relatively pure alfalfa leaf meal was collected from a set of commercial hay bales. This leaf meal contained 29.8% CP. The leaf meal was heated in a forced air oven for 0, 15, 30,

60 or 120 min at 150°C. This temperature was chosen because it is the approximate temperature of the waste heat stream available from a steam turbine electrical generator. Treatments were applied to three separate batches of leaf meal to allow statistical evaluation of the effectiveness of the treatment. The control (0 min heating) and treated leaf meal samples were evaluated for unavailable protein (ADIN), soluble protein content, ruminal rate and extent of protein degradation using nylon bags suspended in the rumen of a dairy cow, and *in vitro* intestinal protein digestion of the residual protein after ruminal incubation. From these measurements intestinally absorbable dietary protein and total tract unavailable protein were calculated.

Results and Discussion

Heating of the alfalfa leaf meal decreased protein solubility and increased the proportion of the protein in the ADIN fraction (Table 1). Ruminal protein degradation was reduced from 64.9% in the control to 53.5% after 120 min of heating. Rate of protein degradation was only reduced at the longest treatment time. Most of the increase in the amount of protein that escaped ruminal degradation was protein that was available for intestinal digestion, as shown by the increased in vitro estimated intestinal protein degradation. The net result was an increase in intestinally available dietary protein after heat treatment. There was no increase in protein wastage as total tract unavailable protein proportion did not change due to treatment.

Our results, along with those published previously by Broderick and co-workers, indicate that alfalfa leaf protein can be partially protected from ruminal degradation. However, this decrease in protein degradability was insufficient to qualify the alfalfa leaf meal as a high bypass protein feedstuff. Generally, the ruminal degradation of bypass proteins is 40% or less. The alfalfa leaf meal only declined to a ruminal degradation of 53.5% after extended heat treatment. This is very similar to the approximately 50% ruminal degradation of the alfalfa hay treated with dry heat or steam. Modification of the heat treatment or addition of some chemical treatment will apparently be necessary for leaf meal to be converted to a bypass protein feed.

While we were unable to convert the alfalfa leaf meal to a high bypass protein source with the heat treatment we utilized, it was decided to determine the economic value of such a product if it could be developed. A diet was formulated using $2.50/bushel corn and $200/ton soybean meal for a cow producing 90 lb of milk/day. Blood meal at $450/ton was included as a competing high protein, high bypass feed (30% ruminal degradation) in a diet formulated to contain 37.5% UIP. The alfalfa leaf meal was assumed to have a ruminal protein degradation of 40% in this scenario. Rations were formulated for 30% CP leaf meal. Under these conditions, the alfalfa leaf meal would increase in value from $123.36/T for the untreated leaf meal to $187.39/T for a bypass protein leaf meal product.

The reader is warned that these economic values for bypass alfalfa leaf meal based on ration formulation for UIP may be overestimates. While the theoretical basis for the importance of bypass protein is well established in dairy cattle nutrition, there is still some concern with how well we have characterized the actual UIP requirement of dairy cattle and how accurate our laboratory methods are at evaluating ruminal protein degradation. Formulating rations on UIP requirements, in addition to those of basic protein, energy and fiber concentrations, often leads to unrealistic feeding programs in terms of cost and practicality. Our suggestion is that the increased value of a bypass alfalfa protein leaf meal product might best be estimated as half of the observed increase derived in this formulation exercise.

Conclusion

Alfalfa leaf meal does respond to heat treatment with an increase in UIP. This decrease in ruminal degradability of alfalfa protein does not appear to result in loss of intestinal availability. Unfortunately the increase in UIP was insufficient to qualify the

heated leaf meal as a high bypass protein supplement. Further work will be needed to determine if another form of heat (i.e., steam), pressurization, and/or addition of a chemical treatment can produce an alfalfa leaf meal product with greater bypass value. Such a product will certainly improve the biomass energy system economics if the leaf meal product can be achieved at a reasonable cost.

Table 1. Effect of heat treatment of alfalfa leaf meal on ruminal protein degradation and in vitro intestinal protein degradation.

	Time of heat treatment (minutes)				
Trait[1]	0	15	30	60	120
ADIN, % CP	3.8[a]	3.3[a]	5.1[ab]	6.7[b]	15.1[c]
Soluble protein, % CP	25.3[a]	22.0[b]	21.0[b]	19.1[bc]	17.6[c]
Ruminal Degradation					
Rate, %/hour	6.9[a]	7.0[a]	6.8[a]	6.2[a]	4.6[b]
Extent, %	64.9[a]	64.0[a]	62.7[ab]	60.1[b]	53.5[b]
Intestinal Digestion					
Extent, %	48.4[a]	50.2[ab]	54.7[bc]	58.9[cd]	60.8[d]
IADP, % CP	16.9[a]	18.0[ab]	20.4[b]	23.5[c]	28.3[d]
TTUP, % CP	18.2	18.0	16.9	16.3	18.2

[1]Intestinally absorbable dietary protein (IADP); total tract unavailable protein (TTUP).

[abcd]Means in the same row not sharing a superscript differ ($P < 0.05$).

In Vitro Ruminal Protein Degradation and Microbial Protein Synthesis on Alfalfa Hay and Silage

V.D. Peltekova, and G.A. Broderick

Introduction

Alfalfa forage conserved as silage or hay is a major dietary component for lactating cows. However, alfalfa protein, particularly that in alfalfa silage (AS), is poorly utilized due to extensive degradation in the rumen. The NRC indicated that undegraded intake protein content of alfalfa hay (AH) was 18% greater than AS. As a proportion of total N, the NPN content of AS typically is more than 50%, while that of AH is about 10%. Results from several studies have shown that protein, not energy, is the first limiting nutrient for milk production in cows fed high AS diets. In lactating dairy cows fed either AH or AS as the sole forage, feeding fish meal increased milk protein yield by 30 g/d and 100 g/d, respectively (Broderick, 1995). Poor performance on high quality AS diets also may result from inadequate capture of degraded N for microbial protein synthesis. Our objectives were to use a new in vitro procedure (Hristov and Broderick,1994) on intact AS and AH, and water soluble extracts and residues from AS and AH, to estimate: 1) microbial protein synthesis and 2) ruminal protein escape.

Materials and Methods

Samples of AS and AH previously fed in two lactation studies (Broderick, 1995) were analyzed for total N, NDF, ADF, and ADIN. Water soluble N and true protein were estimated by the Kjeldahl and Bradford assays. Large samples of ground AS or AH also were extracted with water at 39°C for 1 h, then centrifuged and filtered. Water soluble extracts from AS (ASE) and AH (AHE) plus insoluble residues from AS (ASR) and AH (AHR) were freeze-dried and analyzed for N by Kjeldahl.

Seven protein sources (casein, AH, AS, AHE, ASE, AHR, ASR) then were incubated in vitro using the ruminal inoculum, incubation conditions, level of $^{15}NH_3$ addition, isolation of ruminal microbial pellets, measurement of ^{15}N enrichments in NH_3 and ruminal microbes, replication, statistical analyses, and computations of rates and extents of protein degradation described by Hristov and Broderick (1994). Net microbial N formation (NMN) after incubation (6 h) was computed for each protein using the equations:

$$MN = (TS^{15}N / B^{15}N) X TSN$$
$$NMN = MN_{prot.} - MN_{blank}$$

where MN is microbial N (mg/100 ml), $TS^{15}N$ and $B^{15}N$ are atom % excess of ^{15}N enrichments of total solids N and bacterial N, TSN is total solids N (mg/100 ml), and $MN_{prot.}$ and MN_{blank} are microbial N (mg/100 ml) in protein-added and blank vessels.

Results and Discussion

As observed earlier, NDF and ADF were not different between AS and AH but CP (N X 6.25) in AS was about two percentage units greater than that of AH (Table 1); ADIN content of AH was twice that of AS. Although NPN content of AS was lower than is typical, its NPN content was greater than AH. Water soluble true protein represented 1.2 and 8.4% of total N for AS and AH, respectively. Extensive protein breakdown to NPN as a result of plant and microbial enzymes during ensiling is well known. Drying during haymaking probably also contributed to the nearly 8-fold greater soluble true protein in AH than in AS. Data used to compute ruminal protein degradation rates and estimate ruminal protein escapes are in Table 2. Net NH_3 release from AS and ASE was greater than from comparable fractions of AH. Net NH_3 release was negative for both ASR and AHR indicating net uptake of NH_3 from the medium; net uptake of NH_3 was greater in incubation of ASR than AHR (Table 2). Net accumulation of total AA N was small in all incubations except those with ASE where it accounted for about 16% of degraded N. Despite the fact that net release of N as NH_3 plus total AA from AS and ASE was about two-fold greater, net synthesis of microbial N on AH and AHE was 29 and 43% greater ($P < .001$; Table 2). This indicated that, in our in vitro system, microbial utilization of degraded N from silage, which was largely NPN, was less efficient than utilization of degraded N from hay. In incubations with insoluble residues, with no NPN or soluble protein added to the inoculum, net microbial protein synthesis was greater ($P < .001$) on ASR than on AHR (Table 2). Ruminal escape of protein, estimated for intact AS, was numerically greater than AH (Table 2). Protein escape also was computed using the proportions of total CP in soluble and insoluble proteins in AS (ASE and ASR) and AH (AHE and AHR), and in ADIN, using a 2-compartment model where the ruminal passage rates for soluble and insoluble proteins were set equal to .12/h and .06/h, respectively. Estimated ruminal escapes, computed from mean fractions (Table 1) and rates (Table 2), were 26.2% for AS (21.2% without ADIN) and 35.8% for AH (25.8% without ADIN). If extents of ruminal escape are discounted for ADIN (which may be considered totally indigestible in the intestine), then estimates of available protein from AS and AH are similar to values reported by the NRC of 23 and 28%, respectively.

Conclusions

Net release of degraded N as NH_3, total AA and microbial protein synthesis, quantified from $^{15}NH_3$ incorporation into microbial protein, was used to estimate rate and extent of in vitro degradation of protein in fractions isolated from AS and AH. Microbial protein synthesis on N fractions from AH was greater than on N fractions from AS. Contribution of the NPN in the soluble fraction to estimated ruminal degradation in AS was greater than in AH. Use of a 2-compartment model, based on water soluble and insoluble N fractions assumed to pass with the liquids and solids, to compute ruminal protein escape yielded estimates of 21 and 26% for AS and AH, respectively. Microbial protein synthesis on N fractions from AH was greater than on N fractions from AS.

Table 1. Composition of alfalfa silage (AS) and alfalfa hay (AH).

Item	AS	AH	SE	$P > F^1$
Dry matter (%)	40.5	85.3	1.4	<.001
NDF (% of DM)	37.7	38.3	3.5	.738
ADF (% of DM)	29.0	28.6	3.4	.813
Ash (% of DM)	10.4	9.6	.4	.001
CP (% of DM)	20.1	18.2	1.2	.019
ADIN (% total N)	5.0	10.0	.4	<.001
Water soluble N (% total N)	42.8	24.8	.8	<.001
Water insoluble N[2] (% total N)	52.2	65.2	. . .	. . .
Soluble protein N[3] (% soluble N)	1.18	8.44	.20	<.001
NPN[4] (% soluble N)	98.72	91.56	.18	<.001

[1]Probability of treatment effect.
[2]Water insoluble N determined by difference [100 - (Water soluble N + ADIN)].
[3]Water soluble protein N was determined by Bradford protein assay.
[4]NPN = (Soluble N - True protein N) x 100 / Water soluble N.

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Table 2. In vitro protein degradation of intact proteins and water extracted alfalfa hay and silage.[1]

Source		Net NH_3 N	Net TAA N	Net NH_3+TAA N	Net MN	Total Degraded N	APD (%)	Degrad. rate, k_d (/h)	Estimated escape (%)
		(mg/100 ml)							
Casein (n=16)	Mean	7.10	.28	7.29	5.81	13.13	88.4	.514	23.5
(k_p = .12/h)[2]	(SE)	.19	.13	.19	.39	.29	2.3	.120	1.5
Intact forage (k_p = .06/h)									
AS (n=48)	Mean	1.16	.98	1.82	5.50	7.32	59.0	.156	29.1
AH (n=48)	Mean	.81	.24	.97	7.11	8.00	64.0	.186	26.6
	(SE)	.62	.46	.73	1.60	1.49	11.9	.070	6.6
	P>F[3]	.006	< .001	< .001	< .001	.061	.061	.056	.067
Water soluble N (k_p = .12/h)									
ASE (n=16)	Mean	5.45	2.30	7.75	6.78	14.53	94.7	.650	18.1
AHE (n=16)	Mean	2.74	.57	3.31	9.70	13.01	91.3	.565	21.1
	(SE)	.69	.84	.51	.89	1.08	6.38	.386	6.9
	P>F	< .001	< .001	< .001	< .001	< .001	.152	.539	.227
Water insoluble N (k_p = .06/h)									
ASR (n=16)	Mean	-3.62	.01	-3.61	8.76	5.15	41.2	.089	40.5
AHR (n=16)	Mean	-1.27	.05	-1.21	7.00	5.78	46.3	.105	36.9
	(SE)	.82	.16	.86	.85	.64	5.1	.017	3.6
	P>F	< .001	.496	< .001	< .001	.009	.009	.011	.008

[1]AH = Alfalfa hay, AHE = alfalfa hay extract, AHR = alfalfa hay residue, APD = adjusted degraded protein, AS = alfalfa silage, ASE = alfalfa silage extract, ASR = alfalfa silage residue, TAA N = total amino acid N.

[2]Passage rate, k_p, used to compute ruminal escape of forage N fractions: Estimated Escape (%) = [k_p / (k_p + k_d)] X 100.

[3]Probability of a significant treatment effect.

Evaluation of Several Methane Prediction Equations for Dairy Cows

V.A. Wilkerson, D.P. Casper, and D.R. Mertens

Introduction

Cattle are estimated to account for 95% of the methane emissions of domesticated livestock, and three groups have been targeted by USEPA (1993) for reduction of methane emissions: 1) dairy cows, 2) beef cows, and 3) feedlot cattle. Methane production by ruminants has been predicted using equations involving dry matter intake (Kriss 1930, Axelsson 1949), intake of carbohydrates (Moe and Tyrrell 1979), intake of digestible carbohydrates (Bratzler and Forbes 1940; Moe and Tyrrell 1979), intake and digestibility of diets (Blaxter and Clapperton 1965), and animal size, milk components, and intake and digestibility of dietary components (Holter and Young 1992).

Indirect respiration calorimeters in the Energy Metabolism Unit (EMU) of the Ruminant Nutrition Laboratory at Beltsville, MD have been in operation since 1958 to determine the energy balance of dairy and beef cattle. An extensive data base containing methane production by cattle has been compiled from EMU experiments which can be used to evaluate equations for predicting methane production by Holstein cows. The objectives of this study were to establish an independent data set of methane production by Holstein cows from the EMU data set and to use it to evaluate the predictability of methane production from published equations.

Materials and Methods

Equations used for predicting methane production include one each from: Kriss (1930), Bratzler and Forbes (1940), Axelsson (1949), and Blaxter and Clapperton (1965) and two from Moe and Tyrrell (1979). The approach used by Holter and Young (1992) to predict methane energy involved the use of three equations with each being used for a specific class of dairy cows: non-lactating cows, lactating cows not fed supplemental fat, and lactating cows fed supplemental fat (dietary ether extract greater than or equal to 3.5%).

Sixteen experiments conducted between the years of 1976 and 1992 were included in the EMU methane data set. Experiments used by Moe and Tyrrell (1979) to develop their equations were not included. In addition, balance trials in the methane data set were selected to contain all variables required by the prediction equations evaluated. The data set contained 660 energy balance trials using 195 Holstein cows that were fed total mixed dairy rations that ranged in forage concentration from 40 to 60%.

Results and Discussion

Observed methane emissions averaged 15.7 MJ/d for lactating and non-lactating cows combined (Table 1). The carbohydrate intake equation of Moe and Tyrrell (1979) had a slight systematic bias (slope = -.09) that resulted in small overpredictions at low levels of methane production and underpredictions at high levels of methane production. This equation had the best fit for predicting methane production for the complete data set of lactating and non-lactating cows with the highest correlation coefficient and lowest error of prediction. The carbohydrate digest equation of Moe and Tyrrell (1979) had a constant bias (intercept = .67) that resulted in overpredictions of methane production.

Fractionating digested carbohydrate into components suggested by Moe and Tyrrell (1979) improved predictions of methane production over the single coefficient used by Bratzler and Forbes (1940). The prediction equation of Bratzler and Forbes (1940), based on total digested carbohydrate overpredicted methane production with a systematic bias (intercept = .63 and slope = .06) that increased with level of methane production.

The equation of Holter and Young (1992) exhibited systematic bias that underpredicted methane at low and overpredicted at high methane production. The complex regression equations of Holter and Young (1992) did not improve estimation of methane

production over the carbohydrate intake and digest equations of Moe and Tyrrell (1979).

A systematic bias that overpredicted methane production was observed for the equation of Blaxter and Clapperton (1965), which used intake and digestibility to predict methane production. The correlation coefficient was lower than those observed with the Moe and Tyrrell (1979) carbohydrate intake or digest equations.

The methane prediction equation of Kriss (1949) is based on dry matter intake. A systematic bias with small overpredictions at low levels and large overpredictions at high levels of methane production was observed for the combined lactating and non-lactating cows. The correlation coefficient for the Kriss (1930) equation was similar to the Moe and Tyrrell (1970) equations, but errors were greater as a result of the large overpredictions at high methane productions.

Axelsson (1949) also used dry matter intake to predict methane production, but his equation had a systematic bias that resulted in overpredictions at low levels of methane production and severe underpredictions at high levels of methane production. The equation by Axelsson (1949) used a second degree polynomial for dry matter intake that resulted in predictions with a plateau followed by increasing underpredictions of methane production as dry matter intake increased above the equation's upper limit (9.73 kg/d).

Conclusions

With the exceptions of the equations of Moe and Tyrrell (1979), most equations for predicting methane production overpredict the methane emissions by high producing dairy cows. These equations can lead to erroneous conclusions about the contributions of dairy cows to atmospheric pollution and global warming. The carbohydrate intake equation of Moe and Tyrrell (1979) was the most accurate and precise of the seven equations evaluated for the production of methane by Holstein cows. The carbohydrate variables (cellulose, hemicellulose, and soluble residue) used by Moe and Tyrrell (1979) are relatively easy to obtain and therefore make this equation usable for predicting ruminant methane production.

Table 1. Observed mean, minimum (Min), and maximum (Max) methane production and predicted methane production from seven literature sources with correlation coefficients (n = 660). Also shown are intercepts (B_0), slopes (B_1), and standard errors of prediction of the regression of residuals (predicted minus observed) vs. methane production.

Equation	Mean	Min	Max	r	B_0	B_1	REP
Energy Metabolism Unit	15.7	3.7	30.2				
Moe and Tyrrell (1979), Intake	15.7	6.5	29.4	.91	1.46	-.09	2.4
Moe and Tyrrell (1979), Digest	16.6	6.6	35.1	.92	.67	.01	2.6
Bratzler and Forbes (1940)	17.2	5.4	31.8	.91	.63	.06	2.9
Holter and Young (1992)	14.7	1.1	33.6	.91	-3.60	.16	3.3
Blaxter and Clapperton (1965)	15.9	6.4	27.2	.89	3.56	.21	2.5
Kriss (1930)	18.6	5.9	36.6	.91	-.96	.25	3.3
Axelsson (1949)	9.7	-12.5	14.5	-.36	13.47	.24	3.7

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A Mass-Action Model of Ruminal Function for Estimating the Filling Effect of Forage NDF

D.R. Mertens

Introduction

The NDF-Energy Intake system was developed to combine the physical and physiological concepts of intake regulation into a practical system of formulating rations for dairy cows. In this system, NDF is used to represent the filling effect of the diet when physical fill limits intake and NEL is used to meet the energy demand when physiological mechanisms regulate intake. Although fibrous by-product feeds are adjusted to reflect differences in filling effect, NDF from different forages is assumed to have similar effects on intake. Several experiments have demonstrated that the NDF-Energy Intake system can be used to formulate dairy rations using a variety of forages; however, the utility of the system may be improved by accounting for the chemical, physical, and biological differences in NDF among sources that influence the filling effect of the diet.

The filling effect of NDF is a function of its composition, particle size, rate and potential extent of digestion, lag time of digestion, rate of particle size reduction, and rate of passage. These characteristics of fiber interact to produce a steady-state mass of matter in the rumen that represents the filling effect of the diet. The impact of each variable on ruminal fill can be determined only by using mathematical models. The objective of this research was to: (1) develop a mass-action model of fiber digestion and passage in ruminants that is based on kinetic characteristics that are commonly measured, (2) solve the model analytically at steady-state, and (3) use the model to evaluate the impact of differences in NDF on the filling effect of the diet.

Materials and Methods

Passage of fiber from the rumen was described as a sequential process of particle size reduction and escape, and digestion of fiber was described as a sequential, two-step process representing lag followed by digestion. The model consists of nine compartments that represent large, medium, and small particles in the rumen with indigestible,

potentially digestible but unavailable (uncolonized), and potentially digestible and fermenting fiber compartments within each size group. Post-ruminal digestion of fiber was represented by indigestible, unavailable, and digesting compartments in the intestines. The lag phase of fiber digestion was represented by the process of transforming potentially digestible fiber that is unavailable for digestion to that which is colonized and actively fermenting.

The model assumes that microbial numbers and activity are not limiting in the rumen and that rates of digestion are limited by intrinsic characteristics of fiber. The model requires 21 input variables which include: intake; NDF concentration; proportion of fiber that is potentially digestible; proportion of large, medium, and small fiber particles entering the rumen; rates of particle size reduction from large to medium, large to small, and medium to small sizes; rates of escape from the rumen for medium and small particles; rates of passage through the intestines; rate of transformation from unavailable to fermenting states for each particle size; and rates of digestion for each particle size.

During simulations, intake of NDF was held constant at .30 kg/hr for a 600 kg cow. The NDF concentration, fraction of NDF that was potentially digestible, rate of digestion, and rate of transformation from unavailable to fermenting fractions were 43.7%, .55, .093/h, and .33/h, respectively, for alfalfa hay, and 65.4%, .79, .080/h, and .33/h, respectively, for grass hay. Fractional rates of digestion of NDF were measured on finely ground samples and were reduced to 80% of measured values for medium particles and 60% of measured values for large particles in the rumen. The particle size distribution of ingested particles was assumed to be 65% large particles, 25% medium particles, and 10% small particles. The combination of entering particle size distribution and rates of particle size reduction and passage resulted in a ruminal turnover time for indigestible NDF of 38 h.

Results and Discussion

In other experiments, we observed that the volume of ruminal contents is rarely increased when separated liquid and small particles are mixed with the mass of large fiber particles removed from the rumen. This indicates that small particles contribute little to the filling effect of the diet and may simply fill the void spaces among large particles. Therefore, we postulated that only the mass of large particles determines the ruminal volume that represents the filling effect of a diet. The contribution of the medium particle pool to the filling effect is unknown. The large particle pool and total ruminal pool of NDF estimated by the model were 2.00 and 7.43 kg, respectively, for alfalfa and 1.86 and 5.97 kg, respectively, for grass. This suggests the grass has less filling effect and should result in higher intake than alfalfa, a result that disagrees with typical observations. However, the physical nature of grasses and legumes suggests that they would not have the same particle distribution during ingestion; grasses do not disintegrate into as many small particles as do legumes. Adjusting the entering particle distribution to 70% large, 20% medium, and 10% small particles for the grass resulted in 2.00 kg of large particles and 6.04 kg of NDF in the rumen.

Changing digestion rate of alfalfa by 10% resulted in a 1 to 1.5% change in the large particle pool in the rumen. Similarly, changing the digestion rate of grass by 20% changed the large particle pool by only 3.8 to 4.3%. Halving or doubling the lag time of digestion for both forages resulted in a 2.5 to 4.3% change in the mass of the large particle pool. Increasing or decreasing the potential extent of digestion of alfalfa by 7.5% changed the large particle pool by 1.5 to 2.5%. Likewise, a 15% change in the potential extent of digestion of the grass resulted in a 4.3 to 4.8% change in the mass of large particles in the rumen. Changes in the mass of total NDF in the rumen were similar to those of the large particle pool, with the exception of the potential extent of digestion, which had a larger effect on total NDF than on large particles in the rumen.

Conclusions

It appears that changes in kinetic characteristics will have small effects on the filling effect of NDF.

Potential extent of digestion and rate of particle size reduction have greater impact on filling effect than rate of digestion or digestion lag time. Although the effects of kinetic differences are small, they may have a significant effect on intake when high forage rations are fed or when animals are attempting to achieve high levels of milk production.

Estimating Manure and Nitrogen Excretion by Dairy Herds

D.R. Mertens, V.A. Wilkerson, and D.P. Casper

Introduction

Increases in milk production and size of dairy herds have increased the need for improved whole farm nutrient and manure management. Current manure production and N excretion standards published by the American Society of Agricultural Engineers (ASAE, 1993) apply to lactating cows of average milk production. These standards may not apply to dairy herds of above average milk production, and do not account for manure excretion by nonlactating and replacement animals on dairy farms. More accurate estimates of manure and N excretion on dairy farms may be estimated if relationships between animal and diet characteristics and manure production are identified and used. The objectives of this study were to: define the average manure and N excretion of Holstein cattle based on N balance measurements and compare them to ASAE 1993 standards, develop empirical equations for improving estimates of excretion that use easily-obtainable animal and diet characteristics, and estimate the annual manure and N excretions for Holstein dairy herds with various levels of milk production.

Materials and Methods

A data base containing manure and N excretion measurements was compiled from energy balance trials conducted over the last 30 years at the Energy Metabolism Unit (EMU) at Beltsville, MD and edited to contain only information from Holstein cattle. The EMU nitrogen data base contained 1,994 energy balance trials utilizing 281 Holstein cows that ranged from 2 to 15 years of age and 78 growing Holstein heifers and steers that ranged from .8 to 2.8 years of age. Lactating cows had milk productions typical of a dairy herd producing 7000 kg annually, and diet characteristics were similar to those recommended by the NRC. Nonlactating cows were fed lactating cow rations at restricted levels of intake, and these rations were higher in CP and percentage of concentrate than those typically fed to nonlactating cows. Growing/replacement animals were fed primarily silage rations containing forages that were high in crude protein.

Diets were mixed rations that ranged in forage concentration from 0 to 100%. Forage and roughage sources included alfalfa, corn, and orchardgrass silages, alfalfa, bromegrass, orchardgrass, and timothy hays, corn earlage, beet pulp, and cottonseed hulls. Energy ingredients included barley, corn meal, dried whey, high moisture corn, oats, and wheat bran. Blood meal, brewers dried grains, corn gluten meal, cottonseed meal, distillers dried grains, fish meal, linseed meal, soybean oil meal, sodium caseinate, and urea represented the protein ingredients. Supplemental fat was provided by cottonseed, Megalac® (Church and Dwight, Princeton, NJ), and soybeans.

Results and Discussion

The average amount of total wet excreta for high producing cows in the EMU nitrogen data base (Table 1) agreed with the ASAE 1993 standard of 86 kg/d. However, daily N excretion of the EMU cows was greater than ASAE standards (.55 vs .45). Manure and N excretion per 1000 kg of animal mass was less for nonlactating cows and growing/replacement animals than for lactating cows.

Daily manure and N excretion for lactating cows was influenced by milk production, dietary CP and NDF, body weight, and days in lactation. A quadratic regression model, containing curvilinear terms for dietary N and NDF, and interactions between milk production and dietary N, resulted in little change in excretion estimates for the two lowest levels of production, but produced lower excretion estimates for cows producing 44 kg of milk daily. Days pregnant, dietary CP and NDF, and body weight were significant factors affecting the excretion of manure and N by nonlactating cows. Although statistically significant, days pregnant had little effect on excretion. Dietary composition had a more dramatic effect, with typical dry cow rations resulting in higher production of total manure and feces, but lower losses of total N than lactating cow rations fed to cows at the EMU. Daily excretion of manure and N by growing Holsteins was influenced by body weight and dietary CP and NDF. A quadratic model, containing curvilinear terms for dietary NDF and interactions between body weight and dietary N, resulted in higher estimates of manure and fecal excretion for all replacement animals. However, N excretion was higher for light-weight animals and lower for heavy-weight animals when the quadratic model was used.

Daily milk production levels (22, 33, and 44 kg) selected by the NRC were used to establish a range in annual herd production for illustrating the effects of increased milk production on expected manure and N excretion by dairy herds (Table 2). Equations developed from the EMU nitrogen data set indicated that excretions from lactating cows producing more than 6800 kg of milk annually will be larger than the ASAE 1993 standards. Combining the estimated excretions of lactating, nonlactating, and growing/replacement animals resulted in projected annual manure and N production for the entire dairy herd. The difference between the entire herd and the milking herd reflects the contribution of nonlactating cows and growing/replacement animals to manure and N excretions on dairy farms.

Conclusions

Relationships between animal and diet characteristics (milk production, body weight, days in lactation, days pregnant, NDF, and CP) and manure excretion improve the accuracy of predicting manure production on dairy farms. These relationships predict manure and N excretions that are larger than ASAE 1993 standards when dairy herds average more than 6800 kg of milk per cow annually. These discrepancies can have a serious impact on the planning of manure storage and handling systems for dairy farms.

Table 1. Manure and N excretion for classes of Holstein cattle in the energy metabolism unit nitrogen dataset.

Measurement	High Lactating	Low Lactating	Nonlactating	Growing	ASAE
	kg per 1000 kg of animal mass per day				
Total Manure	89.1 ± 22	69.1 ± 19	36.5 ±12	62.8 ± 13	86 ± 17
Feces	60.1 ± 18	42.4 ± 14	15.8 ± 8	30.2 ± 10	60
N Intake	.79 ± .19	.59 ± .17	.27 ± .11	.49 ± .17	± .10
Total Excreta N	.55 ± .51	.44 ± .16	.25 ± .09	.41 ± .14	.45 ± .10
Fecal N	.27 ± .08	.20 ± .05	.08 ± .03	.18 ± .06	
Urinary N	.28 ± .10	.25 ± .13	.17 ± .07	.24 ± .10	

Table 2. Projected annual manure and nitrogen excretion from a Holstein cow herd.

Milking herd				
Milk, Kg/cow	6800	10200	13700	ASAE[1]
Total manure, Tonne[2]	1477.4	1773.0	1959.7	1600.0
Total feces, Tonne	1018.3	1236.5	1373.2	1116.9
Total excreted N, Tonne	8.4	10.8	12.9	8.4
Total fecal N, Tonne	4.4	5.6	6.7	
Complete herd including nonlactating and replacement animals[3]				
Milk, kg/cow	6800	10200	13700	
Total manure, Tonne	2288.0	2583.5	2770.2	
Total feces, Tonne	1439.9	1658.1	1794.8	
Total excreted N, Tonne	12.5	14.9	17.0	
Total fecal N, Tonne	6.2	7.4	8.5	

[1]ASAE, American Society of Agricultural Engineers. 1993

[2]metric Tonne = 2,200 lbs.

[3]n = 85 milking cows, 15 nonlactating cows, 30 two year old heifers, 25 yearling heifers, and 25 heifer calves.

Particle Size and Moisture Content of Corn Grain and Their Effects on Dairy Cow Performance

T.R. Dhiman, and L.D. Satter

Introduction

Processing of corn grain can improve starch utilization. Reducing particle size increases surface area for microbial attachment and can result in an increase in both rate and extent of starch digestion in the rumen. The objective of this study was to determine the long term effect of feeding either dry shelled corn that was rolled, coarsely ground high moisture ear corn (HMEC), and ground high moisture ear corn (GHMEC) on performance of dairy cows.

Materials and Methods

Thirty-seven mature cows and 34 first lactation cows were assigned before calving to one of three treatments. Cows were fed 50% forage and 50% grain diets (DM basis). The forage portion of the diet was 2/3 alfalfa silage and 1/3 corn silage. The grain portion of the diet contained either dry shelled rolled corn (Trt 1), coarsely ground, high moisture ear corn (Trt 2), or finely ground high moisture ear corn (Trt 3) along with roasted soybeans and soybean meal as protein supplements. The experiment started at calving and lasted until cows completed wk 30 of lactation. Particle size distribution of different corn treatments is given in Table 1. The ingredient and chemical composition of diets (1-6) is given in Table 2. Diets 1, 2, and 3 were fed during the first 16 wks of lactation. After 16 wks, cows were switched to diets 4, 5, and 6. Diets were balanced for protein and fed as TMR once daily. Dry matter and crude protein content of alfalfa and corn silage were 32.7 and 34.3%; 19.4 and 7.4%, respectively. Daily feed intake and milk yield were recorded. Milk samples were analyzed once weekly for composition. Body weights were recorded weekly.

Results

Results are summarized in Table 3. Cows fed diets containing either rolled corn or HMEC or GHMEC had similar feed intakes but trended higher with HMEC and GHMEC. Milk yield (3.5% FCM) was slightly higher ($P = .09$) with HMEC and GHMEC than with dry rolled corn. Milk fat and protein contents were not different among treatments. Feed efficiency was slightly improved with HMEC and GHMEC compared with rolled corn. Even though HMEC and GHMEC contained approximately 8% cob (DM basis), these two forms of corn supported slightly higher milk yields than did dry rolled shelled corn. Yield of nutrients per acre of harvested corn is definitely higher if harvested as high moisture ear corn than if harvested as dry shelled corn.

Conclusion

High moisture ear corn, whether coarsely or finely ground, appeared to support higher milk production than did dry rolled shelled corn. Fine grinding of high moisture ear corn may have had a slight advantage over coarsely ground high moisture ear corn, but the difference was small.

Table 1. Particle size distribution of corn treatments.

	Screen mesh size, mm				
Treatment	4.75	3.36	1.18	0.6	Pan
	% retained on the screen				
Dry shelled rolled corn	9.1	71.5	14.4	2.6	2.3
High moisture ear corn	56.6	28.4	6.9	3.1	5.1
Ground high moisture ear corn	1.8	19.7	23.6	18.1	36.8

Table 2. Ingredient composition of diets, % DM basis.

	Diets					
Ingredient	1	2	3	4	5	6
Alfalfa silage	33.3	33.3	33.3	33.3	33.3	33.3
Corn silage	16.7	16.7	16.7	16.7	16.7	16.7
Rolled corn	34.75	-	-	37.45	-	-
HMEC	-	34.75	-	-	37.45	-
GHMEC	-	-	34.75	-	-	37.45
Roasted soybeans	13.2	13.2	13.2	2.5	2.5	2.5
Soybean meal	-	-	-	8.0	8.0	8.0
Sodium bicarbonate	.25	.25	.25	.25	.25	.25
Dicalcium phosphate	1.1	1.1	1.1	1.1	1.1	1.1
Trace-mineralized salt	.7	.7	.7	.7	.7	.7
Vitamin ADE suppl.	trace	trace	trace	trace	trace	trace
NE_L, Mcal/kg DM	1.64	1.67	1.67	1.62	1.64	1.64
Crude protein	16.0	15.9	15.9	16.0	15.9	15.9

Table 3. Feed intake, milk yield, and milk composition.

Parameter	Treatment: Dry Rolled Corn	HMEC	GHMEC	SEM	*P*
Feed intake, kg/d	20.0	20.5	20.9	.6	.25
Milk yield, kg/d	31.1	32.3	33.2	.7	.2
3.5% Fat-corrected milk, kg/d	32.2	34.0	34.4	.6	.09
Milk fat, %	3.75	3.84	3.75	.1	.6
Milk protein, %	3.14	3.13	3.13	.04	.9
FCM/feed intake, kg/kg	1.63	1.68	1.66	.04	.5
Body weight, kg					
Initial (wk 1)	562	580	580		
Final (wk 30)	602	600	635		
Gain (wk 30-1)	40	20	55		

Omasal Sampling Technique for Assessing Fermentative Digestion in the Forestomach of Dairy Cows

P. Huhtanen, P.G. Brotz, and L.D. Satter

Introduction

Abomasal or duodenal cannulae in sheep and cattle have been used as digesta sampling sites to study fermentative digestion in the ruminant forestomach. Endogenous secretions and unrepresentative digesta samples can introduce variance at these locations. Also, abomasal or intestinal surgery is more difficult than installing a rumen cannula and is more stressful for the cow. The purpose of this study was to determine if a sampling device installed in the third compartment of the cow's stomach could be used to obtain reliable digesta samples for study of rumen fermentation.

Materials and Methods

A device, made from polyvinyl chloride (PVC), consisting of a ring (15 cm o.d., 12 cm i.d. and 1.5 cm thick) fused to a plate (6 cm dia and 1.5 cm thick), was inserted into the omasum via the rumen cannula. Attached to the plate was a sampling tube (1.2 m long, 1.6 cm o.d. and .95 cm i.d.) which went through the rumen to the rumen cannula. This was attached to a pressure/vacuum pump that alternated between 100 mm Hg of vacuum and 50 mm Hg pressure. This alternating action kept the omasal end of the tube from becoming plugged yet resulted in sucking of omasal digesta out of the animal and into a collection jar.

Eight rumen cannulated Holstein cows fitted with rumen cannulae were randomly assigned to two groups [control (C)-without the omasal sampling device and experimental (E)-with the sampling device]. The experimental design was a simple switchback experiment with each period lasting 21 days. The first 12 days were for adjustment, and the last 9 days were for sample collection. The sampling device was inserted at the beginning and removed at the end of each period. The diet contained (DM basis): 50.0 % alfalfa silage, 36.2 % high moisture ear corn, 12.0 % soybean meal, and a mineral and vitamin supplement. Cr-mordanted straw, Yb-labeled maize and CoEDTA were used as digestibility markers, and La-labeled alfalfa silage and Sm-labeled corn were used as digesta passage

markers. Effects of the sampling device on feed intake, milk production, digestibility and digesta kinetics were measured.

Results and Discussion

Control cows had a higher dry matter intake than experimental cows (24.0 vs 21.8 kg/day), but most of this difference occurred during the sampling period. Large amounts of digesta (6 liters/day) were removed, and this may have contributed to the reduction in feed intake. The sampling device seemed to have little effect on feed intake in a subsequent experiment where much less digesta was removed during sampling. Milk yield tended (P = .058) to be smaller for E than C (35.2 and 36.9 kg/cow/day). No differences between C and E cows were noted in the digestibility of the rations. Total mean retention time of La (26.7 vs 31.1 h, P = .05) and Sm (25.1 vs 29.6 h, P = .09) were shorter for C than E cows, possibly because of higher DM intake. Pool size of rumen digesta and digesta DM and particle distribution of rumen digesta and feces were similar for both treatments. There were no difficulties in aspirating digesta from the omasum. Flow of omasal digesta occurred during the reticular contraction and was very slow or nil between contractions. The average DM content of omasal digesta was 45.2 g/kg (CV within cows was 11.7 %). Cr/Co and Yb/Co ratios were lower in omasal digesta than in feces (0.55 and 0.55 vs 0.73 and 0.70), suggesting that digesta samples were not representative of digesta flowing from the rumen. Apparent ruminal OM digestibility (with SE) was 0.430 (0.0174), 0.572 (0.0122) and 0.494 (0.0176) when estimated using Cr, Co and Yb as single markers. Calculating omasal OM flow by a double marker method (Cr/Co) yielded the following: apparent ruminal OM digestibility (0.511, SE=0.0126); apparent OM digestibility in the intestines (0.477, SE=0.0147); and apparent ruminal OM digestion as a proportion of total apparent OM digestion (0.683, SE=0.0151). When calculated with the double marker method, NAN flow as a proportion of N intake was 0.687 (SE=0.0165). The efficiency of microbial protein synthesis using total purines as a microbial marker averaged 21.8 (SE=1.33) g/kg OM apparently digested in the rumen. Apparent degradability in the rumen was 0.643 (SE=0.0151).

Conclusion

Results suggest that the omasal sampling technique can be used to estimate ruminal digestibility and microbial protein synthesis. Studies modifying the technique to obtain a more representative digesta sample are in progress.

U.S. Dairy Forage Research Center
Annual Dairy Operations Report, January 1995

L.L. Strozinski - Herd Manager

The Research Center herd now totals 650 animals. This represents an increase of 30 animals over the past year. We are currently milking 290 cows and selling an all time high of approximately 19,500 pounds of milk per day. The remainder of the herd consists of 30 dry cows and 330 replacement heifers. During the past year our DHIA rolling herd average peaked at 20,182 pounds of milk, 629 pounds of protein and 743 pounds of fat. In 1994 the Center marketed more than 5.7 million pounds of milk.

In February of 1994 we began the use of BST in the herd as a general management practice. Research scientists can decide whether or not animals on their particular trials are placed on the BST protocol. Response to BST has been apparent; however specific response measurements are difficult to make without appropriate controls. In general, cows receiving BST tend to peak higher and maintain higher production throughout lactation. One observation is that in order to provide adequate dry time for the cows, we are drying cows off at higher production levels than we did in the past. No significant increases in health or reproduction problems in the cows on BST were observed. Body conditions of the cows at the end of lactation is a concern. The feeding program has been adjusted to attempt to achieve body condition scores in the range of 3.5-4.0 at dry off time.

During the past year, a total of 320 cows were used in nine nutritional research trials. The trend continues toward longer trials with more animals per treatment.

The economic climate of the dairy industry has brought renewed interest in seasonal calving and grazing. The 1993 grazing trial with replacement heifers was followed by a more intense lactating cow grazing trial in 1994. Fifty-one cows which calved in the spring were rotationally grazed on the plots established immediately north of the field facility. Accommodations were made to allow these cows to traffic through the heifer barn to the milking parlor. After milking, those cows which were assigned to receive supplemental feed were given access to the free stall area which was equipped with Calan gates to facilitate individual feed intake measurement. This was our first experience using the Calan gates. Training a cow to select and operate a gate was a time consuming and patience testing experience. However, once training was complete, the cows operated the gates very well and few problems, other than the increased labor requirement for feeding, were encountered.

Several facility modifications and equipment purchases have taken place in 1994 to facilitate increased capacity and efficiency of the operation.

Two stationary feed wagons have been purchased and strategically installed to hold and dispense research rations. Diets can now be blended in large batches and then dispensed to smaller carts for delivery to the research cattle. This modification results in considerable labor savings as well as reduced wear and tear on the equipment.

Modification of the stalls in E barn has just been completed. The original barn design consisted of two separate sections; one section contained 48 stanchions and the other 12 stanchions. A third section containing 8 stalls was not changed. The two sections were separated by a wall. All stalls were five feet wide. The stanchions and the dividing

wall were removed and seventy-two new 4 1/2 feet wide tie stalls were installed. The total barn capacity was increased by twelve cows. A total of 80 mature cows can now be housed and individually fed in E barn.

Cow comfort, cleanliness and overall health are always of concern. A new concept in this regard is to install mattresses in the cattle stalls. These mattresses consist of ground rubber covered by a woven polyester/nylon fabric. Most of the milking herd stalls have been fitted with these mattresses and supplies are on hand to complete the project this winter. This project may reduce our overall bedding needs and our dependence on an aging manure separator system.

Construction is currently underway to provide additional conference room and employee lunch room space at the Center. Current space in both these areas has been inadequate. The new conference area will have a capacity of 80-100 people. It will enable the Center to host more outreach activities in the future.

We have continued to upgrade the computer capabilities at the farm. Equipment purchases, upgrade modifications and the addition of new software for farm management and accounting have been made. Time to learn, understand, apply and teach these new applications is now a limiting factor in their use.

The field facility continues to function with a fine group of hard working, dedicated, cooperative employees. Their efforts to keep the entire program functioning 365 days a year is commendable.

U.S. Dairy Forage Research Center Annual Field Operations Report, January 1995

R.P. Walgenbach

The 1994 growing season started out being fairly dry with 2.05 inches of rain in April and only 1.51 inches in May. Temperatures were more typical for April and May in this region. The amounts and timing of rains in June, July and August were nearly ideal for growing crops. Any stress due to lack of soil moisture was minimal during this growing season. Growing degree units were somewhat above averages for this area and definitely above the 1993 season. Alfalfa survived the winter season in excellent condition and stands were excellent compared to past years. The most significant weather occurred on July 4 at 3:15 p.m. when a tornado struck Sauk County. This tornado demolished about half of our hay storage shed, tore the roof and doors off the chemical storage building, destroyed several other pieces of equipment and resulted in the loss of one animal. Fortunately, no injuries occurred from this tornado, and it did not affect the ongoing research or farming operation.

The dry weather allowed for early and rapid planting of all crops. Corn planting was started on April 18 and basically finished by May 4. Soybean planting began on May 3 and was basically finished on May 18.

The dry spring also allowed timely applications of liquid manure to crop land that was planted without tillage. It also facilitated the emptying and dredging out of our clay-lined manure storage lagoon. This phase of the earthen lagoon renovation project exceeded my expectations and was accomplished with few problems. New and established stands of alfalfa were in excellent condition going into the winter period. Corn yields were very consistent from field to field this season. Corn yields ranged from 135 to 185 bu/acre. Soybean yields ranged from 41.2 to 73.1 bu/acre and averaged 59.4 bu/acre on 214.5 acres. All soybean fields were infested with a moderate level of white mold which reduced yields 7-12%. As with other crops, the

forage yields were very good this season. Established fields produced nearly 5 tons/acre of dry matter. High moisture barley yields ranged from 50 to 95 bu/acre. Fields yielding 50 bu/acre were underseeded with alfalfa and had less nitrogen applied.

This past season all soybean fields, half of the barley fields, and about 60% of corn fields (187 acres) were no-till planted. The dry early spring influenced herbicide activation which caused reduced weed control in many fields. In some cases, a follow-up treatment of post-applied chemicals and/or cultivation were needed. Some no-till fields are developing problem patches of tough perennial weeds such as hemp dogbane, Jerusalem artichoke, common milkweed, and quackgrass. This past growing season also produced a vigorous crop of dandelions throughout the state, especially in no-tilled fields.

The forage harvest this past season produced a very good quality and very consistent composition of feed. As one might guess, the frequent summer rains caused some problems with the forage harvest. Because of the manure storage lagoon remodeling, we hauled manure on a daily basis this cropping season. We anticipated that the remodeling project would be completed in mid-July; however, it was not completed until early October.

The installation of the electronic gate to replace the manned guard at gate 8 was completed; however, it has not operated very reliably. To correct this, we are changing the gate opening signal from microwave transmission to a telephone line. The video signals will still be sent via the microwave system. This has been a very frustrating project that will hopefully be resolved with phone line signal transmission. The fuel contaminated soil has been remediated and certified as such by the state of Wisconsin Department of Natural Resources. As I mentioned above, the clay and concrete-lined manure storage facility is about 2.75 million gallons. We are all glad that it is finished. We continue to make equipment and facility purchases. This year we purchased a fourth John Deere tractor (85 h.p.) equipped with front wheel assist and a Case IH field chopper. We also have made major overhauls of two of our trucks used for forage and grain hauling. A 10,000 bu capacity soybean storage bin was erected this fall near the new chemical storage building. This has been a very busy and interesting year at the forage center. We have participated in several major remodeling projects, a destructive tornado, new building projects, unplanned breakdowns, the clean-up and rebuilding after the tornado, daily manure hauling, implementation of the pasture study and the harvesting of an exceptional crop. The staff at the field facility continue to perform in an exceptional manner. Their efforts and dedication are appreciated and they deserve much thanks for their contributions to the mission of the research center.

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